Sulfate Reduction in Produced Water via Expanded Granular Sludge-Bed Reactors

By

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Abstract

In 2012, an estimated 890 billion gallons of produced water were generated for all U.S. onshore and offshore oil and gas production sites (Veil, 2015). Reusing produced water seems beneficial for oil companies, but may do more harm than good by inducing scale, such as calcium sulfate scales. This thesis evaluates the potential of using biological sulfate reduction to remove sulfate in a synthetic produced water at 30 °C via two expanded granular sludge bed (EGSB) reactors each with a working volume of 3.3 L, and a reactor pH > 7.5. Propionate was supplied as the sole electron donor and sulfate was added to maintain a COD/SO_4 ratio between 1.5-2.3. Based on the results from the study, high sulfate reduction efficiencies (>90.0%) were achieved at low to moderate salinity levels (10-30 g·L^{-1} of NaCl). At a salinity level of 30 g·L^{-1}, the average sulfate reduction efficiency was 94.0± 1.2%. Rapidly increasing the salinity content from 15 to 40 g·L^{-1} of NaCl resulted in poor system performance (sulfate reduction efficiency of 32%), while increasing the salinity in 5.0 g·L^{-1} increments proved to be an effective method to acclimate the granules to increasing salinity. At higher salinity levels (35-40 g·L^{-1}), the salinity began to affect the system performance, but the effects were reversible. The total dissolved sulfide concentration increased from 90 to 320 mg·L^{-1} of S^{2-} 10 days after lowering the salinity from 40 to 15 g·L^{-1} of NaCl. Results from PHREEQC modeling showed that produced waters commonly encountered in Wyoming's Minnelusa oil formation were susceptible to calcium sulfate scale, and that these systems could greatly benefit from this biological treatment process.

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I. Table of Contents

Introduction

The oil and natural gas industry is developing water reuse technologies due to economic pressures and state-based regulations. Since the 2000s, horizontal drilling (unconventional production) combined with hydraulic fracturing has exploded due its ability to capture shale-gas and oil in source rock that seemed economically infeasible with conventional production methods (vertical wells) (Burden et al., 2016). In the United States, unconventional production of natural gas from shale is expected to increase from 142 billion $m^3 \cdot yr^{-1}$ in 2010 to 385 billion $m^3 \cdot yr^{-1}$ by 2035 (Sieminski, 2013). Technologies utilized for tight shale formations include multi-stage fracturing and hydraulic fracturing. After hydraulic fracturing, the flow in the reservoir is reversed, and the fluid comes out of the injection well. Initially, the water that comes back to the surface during fracturing is identified as flowback water, while any water that comes back to the surface during oil production is identified as produced water.

Hydraulic fracturing requires large volumes of water pumped at high rates, which generate tremendous volumes of flowback water (Aften, 2010). In 2014, the total volume of water generated in the U.S. oil and gas industry was approximately 820 billion gallons (Veil, 2015). This large volume of produced and flowback water is an economical concern for companies where as much as 40-55% of a well's operational and maintenance cost is associated with produced water management and disposal (Dittrick, 2017). The typical produced water management and disposal methods include Class II injection wells, surface storage, treatment and reuse, evaporation ponds, and zero-liquid discharge. Not only is it an economic concern, but federal and local agencies have also identified that deep well injections may be associated with induced seismicity (Buchanan et al., 2014;

EPA, 2015), further pressuring industries to reduce the amount of water being disposed via deep injection wells. Moreover, the average water use per horizontal well was approximately 1.5 million gallons (MG) in 2011, which has further increased to 2.7 MG per well in 2014 (Goodwin et al., 2014). Typically, this is a small fraction of the total water use in a region. However, in some local counties where freshwater supplies are scarce and there is a high number of oil wells, this fraction of water can be > 50% of the total water use in a community (Burden et al., 2016). Due to the cost of transporting water, disposing of produced water, and the pressure from local agencies, companies are actively looking for reuse options for produced and flowback water during operations.

Current disposal methods all have significant drawbacks and some are relatively expensive such as desalination treatment. Still, the most attractive option is to treat and reuse the produced water. However, the chemical composition of the produced water having high concentrations of total dissolved solids (TDS), and potentially containing radioactive compounds and toxic compounds including BTEX compounds, often limit the reuse applications. For example, irrigation with produced water is often restricted by the high concentration of sodium ions, so the water must be either blended or treated before reuse.

Reusing produced water seems advantageous for oil companies but poses many challenges for optimizing production. Interactions between waters, such as the formation water and injection water, may significantly increase scaling potential in the system, making them incompatible for blending. Scaling is one of the biggest obstacles in the oil and gas industry to overcome, which has been linked to equipment erosion and flow

restrictions resulting in higher pumping rates. In addition, scale formation may also cause damage to the injection or production wells (Moghadasi et al., 2004).

Produced water composition varies significantly across the U.S. and is largely dependent on the type of formation and the minerals present in the formation rock (Otton and Mercier, 1995). In Wyoming, many formations contain sulfate salts, correlating to the formation water containing relatively high concentrations of sulfate (above 1,500 mg/L) from the dissolution of anhydrite and gypsum. Produced waters high in sulfate may lead to calcium sulfate scaling issues in the formation and on surface and subsurface equipment.

Removing scaling constituents such as sulfate often requires expensive treatment technologies. For membrane treatment technologies, including reverse osmosis, the energy requirements alone, about 3.5-6.0 $kWh \cdot L^{-1}$, make this treatment alternative very expensive (Subramani and Jacangelo 2014; Kaplan et al., 2017). This has led investigators to consider using biological sulfate reducing bacteria to minimize the scaling potential of sulfate salts in produced water. Sulfate reducing bacteria (SRB) have been successfully cultivated in bioreactors to treat sulfate-rich wastewaters (van Houten et al., 1995; Vallero et al., 2004; Liu et al., 2010; Pérez et al., 2018). Different combinations of important experimental parameters can be varied to favor SRB growth and to enhance sulfate reduction including temperature, electron donor(s), COD/SO4 ratio, pH, and bio-reactor type (Jeison et al., 1999; Lens et al., 2002; Muyzer et al., 2008; Hao et al., 2014). In addition to these parameters, only a few studies (Vallero et al., 2004; Vallero et al., 2005) have investigated sulfate reducing bacteria in a saline environment. Typically, in these studies, NaCl was the only salt added to simulate salinity. To the author's

knowledge, the study described herein represents the first time a sulfate reducing bioreactor was utilized to treat synthetic produced water containing other salts such as KCl, $CaCl_2$, and $MgCl_2 \cdot 6H_2O$. .

The ability to predict scaling and the chemistry occurring in the geologic formation is a challenge in the oil and gas industry, but it is considerably important in reservoir management (Hoang et al., 2007; Moghadasi et al., 2004; Moghadasi et al., 2006; Naseri et al., 2015). PHREEQC is a free public domain software with a thermodynamic computer model that has the capability of determining water chemistry within a reservoir (Parkhurst et al., 1999). In past studies (Thyne and Brady, 2016), PHREEQC has been used to determine reservoir pH and predict scaling potential in oil and gas reservoirs.

The objectives of this study are to investigate biological sulfate reduction in a synthetic produced water at various salinity levels and to quantify the scaling potential of calcium sulfate in produced waters commonly found in Wyoming's Minnelusa formation via PHREEQC.

Background

A. Deep well Injection for Brine Disposal - Induced Seismicity

Produced water reuse in the oil and natural gas industry is growing due to the large volumes of produced water being generated from unconventional oil and gas production associated with hydraulic fracturing. Typically, the large volumes of brine waters are being disposed of via deep well injection. However, deep well injection of such large volumes of water may cause induced seismic activity. The U.S. Geological survey uses the Modified Mercalli Intensity Scale to assign intensities to earthquakes. The intensity scale

is not designed on a mathematical basis but based on the observed effects during each earthquake. The lower numbered earthquakes represent a low intensity earthquake, while higher numbered earthquakes have larger intensities and cause more severe destruction. The magnitude of an M1 is 1.0, and typically not felt by humans. A M3 earthquake is felt by people if indoors and has magnitude of 3.0-3.9. (USGS, 1989). **Figure 1** (USGS, 2019) shows that there has been a substantial increase in M3 earthquakes recorded in the central and eastern part of the United States since 2009.

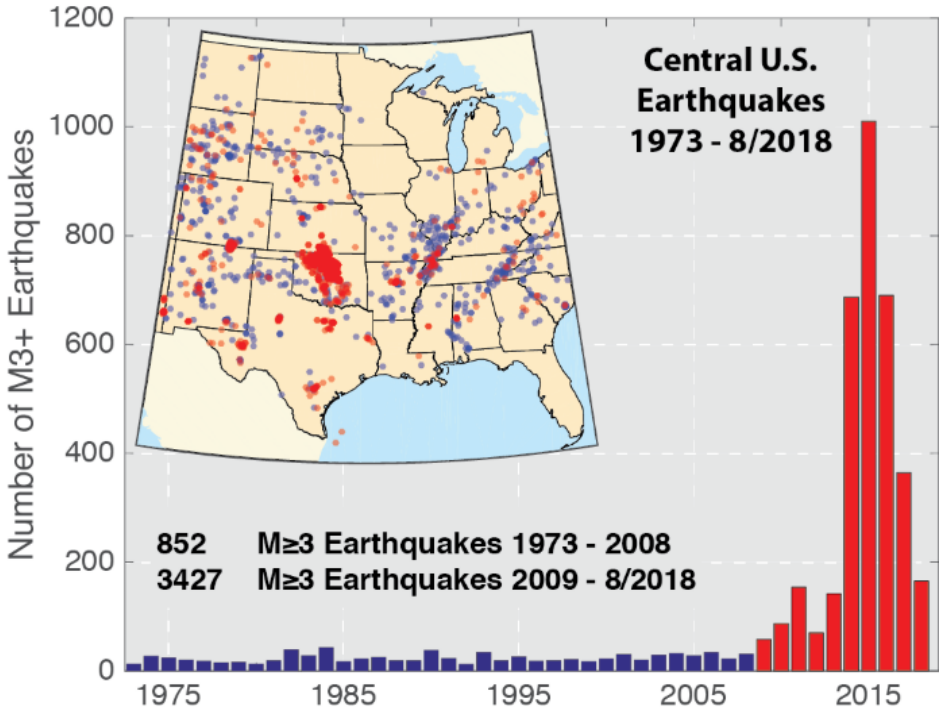

Figure 1. Annual number of earthquakes with a magnitude of 3.0 or larger, 1970-2016 (USGS, 2019).

In addition, there has been a significant increase in M3 + earthquakes in southern Kansas stretching into Oklahoma. A possible explanation of the spike of earthquakes in this region is the increase in oil and gas production and the corresponding increase in the volumes of brine water being deep well injected in the region (Buchanan, et al., 2014).

State and federal initiatives and guidelines have been created to combat induced seismic activity associated with Class II disposal wells. In 2015, the EPA wrote a report to address the issue of the possibility of injection fluids migrating in Class II wells from induced seismicity (EPA, 2015). The report did not state any formal guidelines, but provided practical management tools and best practices techniques to the U.S. Underground Injection Control (UIC) director in the region (Folger and Tiemann, 2014). Other guidance for reducing the seismic activity induced from Class II injections wells include recommendations from the National Research Council and other state agencies. The recommendations were adapted from "the U.S Department of Energy to address induced seismicity associated with enhanced geothermal systems" (Buchanan et al., 2014). In order for these recommendations to work, industries, state agencies, and the research community would have to work together to appropriately monitor, collect data and enforce protocols (Buchanan et al., 2014). Pressures from federal and state agencies to reduce volumes of wastewater generated further encourages operators to incorporate produced water reuse in daily operations (Folger and Tiemann, 2014).

B. Scaling in the Oil and Gas Industry

Produced water has been incorporated in the base fluid for hydraulic fracking fluids for decades, but the primary use of produced water is secondary oil production, specifically in water flooding applications (Merdah et al., 2008). Reusing produced water may be the most cost-effective approach to disposal; however, interactions between incompatible waters may significantly increase the scaling potential in the system. The type of scale formed depends on a variety of parameters including pH, pressure,

temperature, the type of oil recovery process, organics, and brine composition (Lu et al., 2012; Freyer, and Voigt, 2003).

One of the most common scales encountered in the oil and gas industry is calcium sulfate (Dyer and Graham, 2002; Freyer and Voigt, 2003). In Wyoming, many formations are classified as Minnelusa for being mainly comprised of limy sandstones with secondary rocks including evaporites, such as carbonate and sulfate salts (Warren et al., 2010). With concentrations of calcium and sulfate exceeding 1,500 mg/L and with temperatures exceeding 100°C at average well depths between 7,000 to 11,000 feet (Mack and Duvall 1984), the formation water is usually in equilibrium with anhydrite. Other common scales found in oil fields are shown below in **Table 1**. Typically, three principle mechanisms are responsible for the formation of scales:

1. A decrease in pressure and or increase in temperature, which is common to calcium carbonate precipitation.
2. The mixing of two chemically incompatible brines that induce precipitation.
3. Brine evaporation increasing the salt concentration above the solubility limit, resulting in salt precipitation.

Table 1. Most common scales in oil fields. (Moghadasi et al., 2006)

Name	*Chemical Formula*	*Primary Variables*
Calcium carbonate (Calcite)	$CaCO_3$	Partial pressure of CO_2, Temperature, Total dissolved salts
Calcium Sulfate: Gypsum (most common) Hemihydrate Anhydrate	$CaSO_4.2H_2O$ $CaSO_4.½H_2O$ $CaSO_4$	Temperature, Total dissolved salts, Pressure
Barium Sulfate	$BaSO_4$	Temperature, Pressure
Strontium Sulfate	$SrSO_4$	Total dissolved salts
Iron Compounds: Ferrous Carbonate Ferrous Sulfide Ferrous Hydroxide Ferrous Hydroxide	$FeCO_3$ FeS $Fe(OH)_2$ $Fe(OH)_3$	Corrosion, Dissolved gases, pH

In calcium sulfate precipitation, the main mechanisms responsible for scaling are from pressure, temperature changes, and high TDS concentrations with calcium and sulfate levels being at supersaturation conditions for $CaSO_4$. Other chemical parameters, such as pH, have little influence on CaSO4 precipitation as the formation of sulfate scales is relatively pH independent (Olajire et al., 2015).

D. Scale Management Techniques

Scale management is vital in maintaining an efficient production well. Scale management techniques vary from site to site and depend on the scheme and phase of oil production, along with the minerals present in the geological formation. In offshore facilities, a common practice is to use seawater as the injection fluid during secondary oil production. Sulfate levels in seawater are around 2,700 mg/L. To reduce the potential of an injection water to form sulfate scales, many large production facilities use a sulfate

removal plant, which incorporates membrane filtration; however, this requires a very large capital investment.

Chemical treatment via scale inhibitors is also a common technique to reduce scaling. Chemical inhibitors do not remove the scaling constituents but reduce or inhibit the rate of nucleation or crystal growth. In certain situations where the injection fluid is high in scaling compounds, scaling inhibitors will only be effective at high concentrations. This option is expensive and may pose an environmental concern because some scaling inhibitors, such as organic phosphates, are very toxic. Other methods to reduce the scaling potential are good operational strategies including minimizing large pressure drops along the production well (Olajire et al., 2015).

Once the scale forms, it becomes costly to remove with mechanical or chemical removal methods, and for some scales, including barium sulfate, it is nearly impossible to remove. Removing scaling constituents in the injection water is usually the best scale management technique. Typically, scaling compounds are removed through membrane filtration, more specifically by nanofiltration (Boczkowski et al., 2015). Membrane technology is effective but very expensive, and new, cost-effective technologies are needed for scale prevention in produced water treatment.

As mentioned earlier, sulfate scales are one of the most common scales encountered in the oil and gas industry. Other industries or situations that involve high inorganic sulfate waste streams include paper mills, tanneries, acid mine drainage, flue gas scrubbing waters, and waters contaminated from seawater intrusion (Lens et al., 2002). Wastewaters high in suflate have been successfully treated by anaerobic bioreactors via sulfate reducing bacteria (van Houten et al., 1995; Dries et al., 1998; De

Smul et al., 1999; Vallero et al., 2004; Liu et al., 2010; Pérez et al., 2018). Typically, in water systems, sulfate reduction is an unwanted biological pathway due to the formation of sulfide, which is highly corrosive and toxic. However, if designed and operated properly, sulfate reduction coupled with a sulfide removal step can be an effective produced water treatment method for removing sulfate. The technologies commonly used to remove sulfide include sulfide stripping, chemical precipitation, and biological oxidation of sulfide to elemental sulfur (Lens et al., 2002).

E. Sulfate Reducing Bacteria Metabolic Pathways

Sulfur is one of the most abundant elements on the planet, and it is a vital nutrient for microbial and plant growth. In the natural environment, sulfur can be found in the earth's crust as pyrite (FeS_2) and gypsum ($CaSO_4$), and is relatively abundant in seawater as sulfate (Hao et al., 2014). In addition, sulfur may be introduced to natural systems through anthropogenic sources including pharmaceutical production, food production, pulp and paper, tannery, petroleum, and mining industries. Sulfur has a wide range of oxidation states from -2 to +6, which in turn creates a complex system that can be biologically converted by a number of pathways as seen in **Figure 2**.

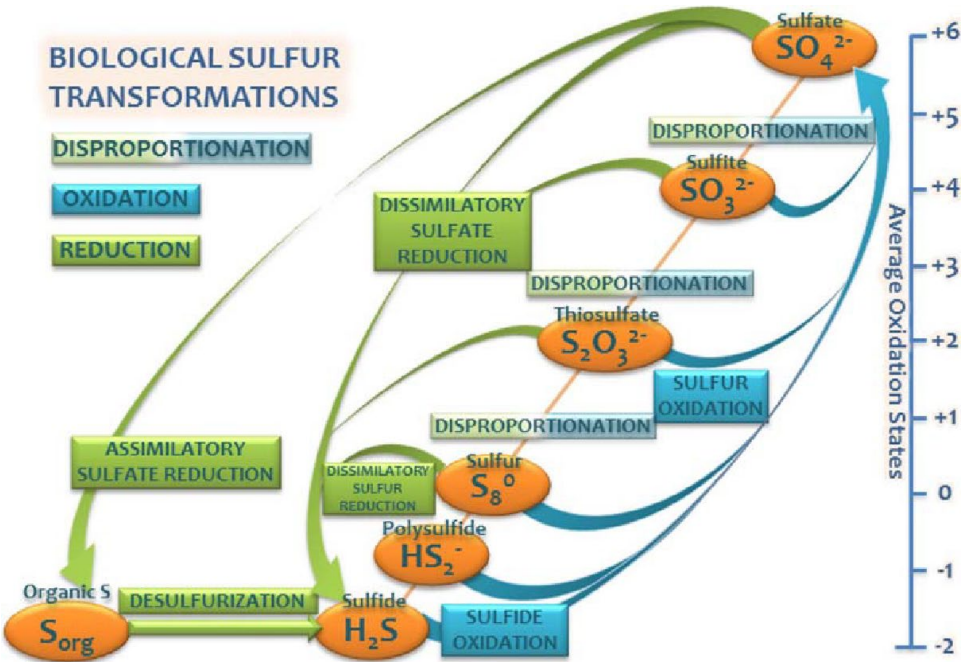

Figure 2. Biological Sulfur Transformations (Sánchz et al., 2014).

In anaerobic environments, sulfate will be utilized as the terminal electron acceptor through the degradation of organic or inorganic matter by sulfate reducing bacteria and/or archaea through anaerobic respiration. The anaerobic respiration process completed with sulfate is generally completed in three steps (Muyzer and Stams, 2008). First, sulfate is activated to adenosine-phosphosulfate (APS). Sulfate is activated due to the high standard electrical potential from redox couple of sulfate-sulfite ($E^{o'}$ of -516 mV). The reaction is too negative for the electron mediators to complete the reduction of sulfate and is why sulfate first must be activated. The activation of sulfate is completed by ATP sulfurylase resulting in APS. Second, the redox couple of APS-sulfite is much more favorable ($E^{o'}$ = -60 mV), and the reduction of APS to sulfite proceeds. Lastly, sulfite is reduced to the final product of sulfide.

Sulfate reducing bacteria (SRB) are named for their ability to reduce sulfate, but in many cases where sulfate is not present, SRB will use other electron acceptors to complete their respiration process such as thiosulfate, sulfur, and sulfite, with all being transformed to sulfide. Some SRB species will even convert nitrite and nitrate to ammonium (Muyzer and Stams 2008; Hao et al., 2014). Other SRB species have been reported to use ferric iron and fumarate as electron acceptors (Barton and Hamilton, 2007; Robertson et al., 2001). Although SRB are anaerobic organisms, some species of SRB are oxygen tolerant and can survive short periods of elevated oxygen levels (Muyzer and Stams et al., 2008).

As mentioned above, the electron donors for SRB are diverse, and SRB can be heterotrophic or autotrophic. Heterotrophic SRB are classified into two groups: ones that completely oxidize organic compounds into CO_2, and ones that incompletely oxidize organic compounds to acetate (Muyzer and Stams, 2008). Autotrophic SRB rely on carbon dioxide as the carbon source and oxidize hydrogen as the energy source. Moreover, studies have shown that hydrogen is a sufficient energy source in completing sulfate reduction, and several SRB strains can utilize hydrogen as an energy source, creating a diverse consortium of SRB species in a hydrogen gas-rich environment (Van Houten et al., 1995; Esposito et al., 2003; Hao et al., 2014). **Table 2** shows viable electron donors that have been studied in biological sulfate reduction systems.

Table 2. Electron donor sources for biological sulfate reduction (Hao et al., 2014)

Electron Donor	Pro (+)	Con (-)
H2/CO	+ Low cost +Most SRB can use it +No organic residual in effluent	-CO toxicity towards SRB -Competition between SRB and methanogens -Hydrogen safety requirements
Synthetic Gas (H2+CO2+CO)	+Low cost +Some SRB have a much higher tolerance for CO	-Availability may be limited
Methanol	+Relative low cost +SRB can dominate in thermophilic conditions	-Methanogens dominate in mesophilic conditions -Only few a few SRB strains can utilize methanol
Ethanol	+Relatively cheap +Easily converted by SRB	-Low biomass yield -Incomplete oxidation to acetate
Formate	+Produce less acetate during formate utilization +Many SRB species that grow on formate, can also grow on H2	-Methanogens outcompete SRB at thermophilic conditions
Acetate		-Methanogens can outcompete SRB for acetate -Only a few SRB can oxidize acetate -Low biomass yield
Lactate	+A wide spectrum of SRB can grow on lactate +Generate large amount of alkalinity +Relieves the sulfide toxicity +Preferable carbon source for SRB	-High cost
Benzene/benzoate	+Can be completely oxidized to CO_2	-Long degradation time (HRT = 264 days) -Cannot be used by some SRB species

Electron donor sources for biological sulfate reduction include amino acids, volatile fatty acids, or a mixture of a synthetic gas consisting of H_2 +CO_2 +CO. In addition, SRB have been found to degrade aromatic compounds including BTEX compounds, specifically benzene, and toluene (Robertson et al., 2001; Plugg et al., 2011). As seen in **Table 2**, selecting an electron donor is an important factor in designing a successful biological sulfate reduction system and in selecting SRB over other anaerobic species.

Sulfate reducing bioreactors are known to require an acclimation period, which may take several months. There are several factors that promote a faster acclimation period, such as selecting an appropriate electron donor. Muyzer and Stams (2008) reported that no methanogens have been identified to grow on organic acids, such as lactate, propionate, and butyrate. Lactate was the ideal choice for an energy source as many SRB species can metabolize lactate in anaerobic digestion. Lactate degradation produces a considerable amount of alkalinity, which in turn reduces the toxicity from the production of sulfide in the system. For every one mole of lactate digested there will be 3 moles of bicarbonate generated. Furthermore, the production of alkalinity is advantageous in maintaining system pH, requiring no pH stabilization. Lactate is the preferred energy source, but it is relatively expensive. Propionate can be a by-product of lactate fermentation, and in anaerobic environments, propionate will generate alkalinity, similarly to lactate fermentation, as seen by the reaction below.

$$C_3H_5O_2^- + 3H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 3H_2 + H^+$$

According to Hao et al. (2014), one should consider the following: the biological sulfate-reduction process selected and the target removal efficiencies of sulfate; the competition between methanogens and SRB for different substrates; the wastewater

characteristics; and the availability and cost of the electron donor. After considering all of these for this study, propionate was selected as an electron donor due to methanogen's low affinity for it and because it generates alkalinity.

F. Optimizing Biological Sulfate Reduction.

Other factors in optimizing sulfate reduction and favoring SRB growth include inoculum, temperature, pH, COD/SO_4 ratio, bioreactor type and operation controls.

i. Inoculum

As mentioned before, starting a bioreactor with anaerobic methanogenic sludge and switching the bacterial community to an SRB-dominated community can take months to years, depending on a combination of factors. To overcome this obstacle, a system may be inoculated with a pure culture of SRB. Omil et al. (1997) and Vallero et al. (2003) bioaugmented pure SRB cultures into an up-flow anaerobic sludge bed (UASB) reactor system. To avoid biomass washout, the regular system operation of flow-through mode was adjusted to batch mode. After normal system operations were reestablished, both studies concluded that bioaugmentation was unsuccessful due to the inability of the inoculant to colonize in the sludge bed, so the SRB eventually washed out of the system. In a later study, Vallero et al. (2005) recorded successful bioaugmentation in a submerged anaerobic membrane bioreactor system (MBR). The MBR process design does not rely on granulation or good setting characteristics. The biomass in MBRs can be in suspension, flocs or attached growth. As a result, washout is not a concern in submerged anaerobic MBR systems. In UASB and expanded granules sludge bed (EGSB) reactors, bioaugmentation of pure SRB cultures is a challenge and is often not achievable, but for

other anaerobic bioreactors that do not rely on superficial up-flow velocities, bioaugmentation is possible.

Another approach to influencing the number of SRB present in a bioreactor system is selecting a seed sludge that already contains SRB. One should select anaerobic sludge that routinely treats an industrial waste stream high in inorganic sulfate. The seed sludge is vital in system performance and shortening the acclimation period (Sánchez et al., 2014). The ideal seed sludge should contain SRB strains that are readily adaptable to bioreactor characteristics, including pH and temperature. For example, a seed sludge adapted to acid mine drainage with a pH around 2.0 would not be the best suited for system designed around a neutral pH.

In any biological system, temperature is always an important consideration. High sulfate reduction rates have been recorded in mesophilic conditions (Dries et al., 1998; La et al., 2003; Vallero et al., 2004; Liu et al., 2010) and in thermophilic conditions (Vallero et al., 2003). SRB have been found to thrive in a temperature spectrum ranging from 0-100°C (Stams and Muyzer, 2008). In addition, the competition between different anaerobes for various electron donors is greatly influenced by temperature (Hao et al., 2014). In methanol-fed anaerobic bioreactors (Weijma et al., 2002; Vallero et al., 2003), it was concluded that SRB outcompete methanogens at temperatures ranging from 50-70°C, while methanogens dominate at mesophilic conditions. For other electron donors including formate, Bijmans et al. (2008) found that only at a temperature range of 65-75°C did methanogens dominate in the system.

SRBs can thrive in extreme environments such as acid mine drainage, with pH values as low as 2, and soda lake sediments, such as the Great Salt Lake located in Utah, can have a pH value as high as 11 (Foti et al., 2007). As with many other physical-chemical properties of bioreactors, the optimum pH is dependent on the sulfur removal process and wastewater characteristics. For anaerobic digestion of sulfate to sulfide, Hao in 2014 indicated a pH range of 4-8 is the typical range observed in literature. In sulfate reducing systems, the pH level may need to be increased to reduce sulfide inhibition caused by undissociated H_2S. Pokorna et al. (2015) reported the sulfide concentration able to inhibit the growth of an SRB population by 50% (IC_{50}) was 30-250 $mg \cdot L^{-1}$. To reduce the percentage of undissociated hydrogen sulfide, Dries et al. (1998) and Chen et al. (2008) increased the pH in the systems to around 7.5-8.0. This alleviated the inhibitory effects of undissociated H_2S. Looking at the ionization fractions of sulfide in freshwater conditions, increasing the pH from 7 to 8 will reduce the fraction of undissociated H_2S from 50% to 9%.

ii. COD/SO_4 ratio

Another operational parameter that influences the reactor performance is the COD/SO_4 ratio. The COD/SO4 can be determined by the organic loading rate (OLR) over the sulfate loading rate (SLR). Having a low COD/SO4 or an excess amount of sulfate in the system favors SRB growth to compete with methanogens and homoacetogens for common substrates such as H_2 and acetate. It was found that at low COD/SO4 ratios, sulfate reduction predominates over methane production (Pokorna et al., 2015), and this relationship can be further increased by decreasing COD/SO4 ratio. De Smul et al. (1999) studied the effect of varying the COD/SO4 in an EGSB reactor system fed with ethanol.

Initially, the COD/SO4 ratio was arbitrarily set at 7.0, and over time, this was reduced to 6.0, 4.3, 3.6, and 2.6. The highest sulfate removal efficiencies were observed at COD/SO4 ratios between 4.3 and 3.6, and the performance drastically dropped at a COD/SO4 value of 2.6. Whereas, Vallero in 2003 observed a different relationship at thermophilic conditions where the sulfate removal rate decreased in methanol-fed up-flow anaerobic sludge bed (UASB) reactors as the COD/SO_4 decreased. It was hypothesized that the poor sulfate removal efficiency at higher COD/SO4 ratios was likely due to the immobilization of SRBs in the sludge bed, but the bacterial community structure was not discussed. SRBs may have been out-competed in this system by methanogenic bacteria. This was one of the few studies to observe this trend where others (Weijima et al., 2002; Vallero et al., 2004) observed high sulfate reduction rates at low COD/SO4 ratios of 0.34-0.5. The reason Vallero et al. (2003) observed this trend in the COD/SO_4 ratio may be partly due to the temperature and substrates used during the experiment. At thermophilic condition, Vallero et al. (2003) reported that methanogenic bacteria outcompete SRBs for methanol, while De Smul et al. (1999) described a reduction in the sulfate reduction performance as the temperature was stepwise increased from mesophilic conditions (33°C) to thermophilic conditions (50°C). Overall, an excess amount of sulfate (low COD/SO_4) favors SRB growth, but the COD/SO_4 values where there is competition between SRB, acetogens, and methanogens depends on the electron donor used to complete the biological sulfate reduction process and on reactor temperature.

iii. Bioreactor type and operational parameters

Many of the biological sulfur conversion technologies used in domestic wastewater applications stemmed from industrial treatment processes. Sulfur conversion

technologies usually occur in one or two stage treatment processes. In acid mine drainage streams with high metal concentrations, the goal is to maximize sulfide production to precipitate as much of the heavy metals as possible (Fe, Zn, Ni, Cd, Cu, Pb). Typically, this is performed in two stages where sulfate reduction occurs in one reactor, and the effluent is sent to another vessel to stimulate sulfide precipitation at controlled conditions. Another sulfate reduction system setup is to perform both operations in one reactor. Sánchez et al. (2014) investigated the one reactor configuration to optimize metal precipitation and crystal growth. It was concluded that the high sulfide level in a reactor performing sulfate reduction will induce larger precipitates with better settling characteristics. Other scenarios, such as waste streams from fermentation or a pulp and paper industry containing high levels of sulfate and unacidified organic matter, provide an opportunity to utilize two-stage anaerobic treatment. Typically, sulfate reduction will be performed in the first stage, and methanogenesis will be selected in the second stage, with the assumption that sulfide is efficiently removed before the second stage. The number of stages is dependent on the treatment goal, but operation and maintenance costs increase as the number of stages increases.

The flow schemes in sulfate reducing bioreactors may vary from batch mode to semicontinuous and continuous. Continuous flow through mode is the most used in sulfate reducing bioreactors (Sánchez et al., 2014). The biological growth may also be attached or suspended growth in continuous systems. **Figure 3**, shows the most common types of bioreactors used in anaerobic digestion for continuous flow through modes.

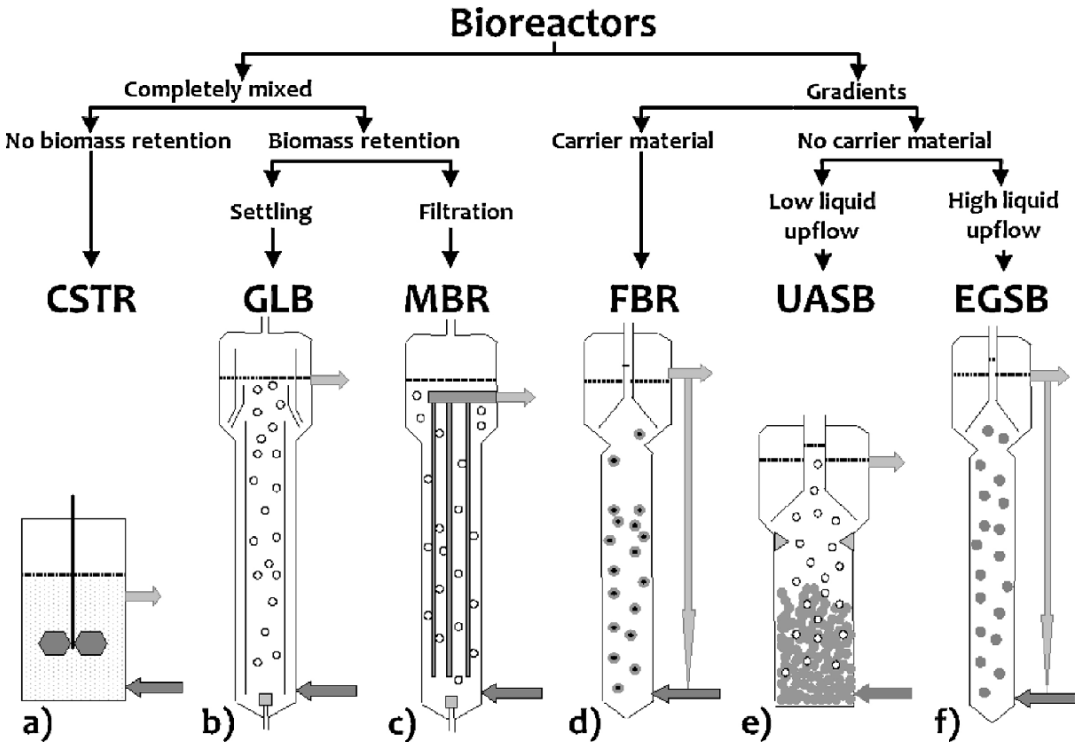

Figure 3. Continuous flow bioreactors used in anaerobic digestion: a) Completley Stirred Tank Reacotor (CSTR); b) gas-lift bioreactor (GLB); c) submerged membrane bioreactor (MBR); d) fluidized bed reactor (FBR); upflow anerobic granular sludge bed (UASB) bioreactor; expanded granular sludge bed (EGSB) bioreactor (Sanchéz et al., 2014).

In this study, two EGSB sulfate reducing reactors were used to determine the effects of stepwise increasing the salinity in a synthetic produced water with propionate as the sole energy source. EGSB systems are similar to UASB, except with a single difference in the upflow velocities. Typically, UASB systems are classified by superficial up-flow velocities of ≤ 1 $m \cdot hr^{-1}$, while EGSB systems have higher superficial up-flow velocities ranging from 3 $m \cdot hr^{-1}$up to 10 $m \cdot hr^{-1}$ (Dries et al., 1998; Jeison and Chamy, 1999). The advantages and disadvantages for EGSB and UASB reactors can be seen below in **Table 3** and **Table 4**, respectively. UASB reactors can handle high organic loading rates and can create high sludge concentrations, while EGSB reactors reduce

dead spots in the system, and will dilute the toxicity of compounds that are biodegradable to a greater extent than UASB reactors (Seghezzo et al., 1998).

In sulfate reducing reactors, as methanogenic bacteria are out-selected by SRBs, there is a biogas evolution that occurs. This transition of biogases has been reported to reduce the external diffusion resistance of granular sludge. Therefore, the mass transfer rates are usually low in sulfate reducing reactors (Huisman et al., 1990). To increase the mass transfer rates in these systems, one can increase the superficial up-flow velocity in the system (Lens et al., 2002). Based on the produced water characteristics containing toxic BTEX compounds and the overall mass transfer limitations in sulfate reducing reactors, the EGSB reactor configuration was selected.

Table 3. Characteristics of expanded granular sludge bed (EGSB) reactors

Characteristics of EGSB Reactors (Seghezzo et al., 1998)	
Advantages	● Higher up-flow velocities (4-10 m/h) ● Higher organic loading rates (up to 40 kg COD m-3d-1) ● Sludge bed is expanded, mostly granular, very active, and promotes good settleability. ● Mixing pattern is different than UASB, with higher up-flow velocities and increased gas production, leading to higher wastewater contact time with the biomass. ● Soluble pollutants are efficiently treated ● Suitable for the treatment of biodegradable toxic or compounds
Disadvantages	● Hydrostatic pressure at bottom will be higher than in a short reactor such UASB reactor, which will affect the performance of reactor. ● Suspended solids are substantially removed from wastewater and will be washed-out of the reactor

Table 4. Characteristics of upflow anaerobic sludge bed (UASB) reactors

Characteristics of UASB Reactors (Seghezzo et al., 1998)	
Advantages	● High sludge concentration ● Low cost of equipment ● High organic loading rates
Disadvantages	● Does not handle toxic/inhibitory compounds as well as EGSB ● May contain dead spots in the reactor

G. Sulfate Reduction in Industrial Saline Wastewaters

The need to treat and reuse industrial saline wastewaters is growing as disposal regulations are becoming stricter and as freshwater sources are dwindling. Common technologies applied in treating saline wastewaters are relatively expensive and require considerable amounts of energy. This is especially true in the oil and gas industry, where common discharge methods are under scrutiny, and the most common method for removing scaling constituents, such as sulfate, is nanofiltration. This is why there is such a need in the oil and gas industry for more economical treatment solutions. One such treatment technology includes utilizing sulfate reducing bacteria to treat produced water generated by the oil and gas industry. High salinity concentrations typically inhibit many biological pathways, but the impacts of salinity on biological processes may be subdued by employing appropriate operational startups and coping strategies.

Typically, salinity levels as low as 10 g·L^{-1} of NaCl inhibit many biological pathways (Kugelman and McCarty, 1965) For example, stepwise increasing the salinity over time has been shown to be an effective method to acclimate aerobic granular sludge to high salinities >80 g·L^{-1} (Wang et al., 2017; Ou et al., 2018). These same principles of gradually increasing the salinity to minimize the effects of salinity could be applied to anaerobic granules as seen in Vallero et al. (2004). The authors investigated the performance of three sulfate reducing reactors at different startup conditions with varied NaCl levels. The startup conditions included (1) starting at a low NaCl level (10 g·L^{1-}) with flow-through mode operation and then stepwise increasing the salinity to 50 g·L^{1-} in 50 days; (2) starting at a high NaCl level of 50 g·L^{-1} in batch mode; and (3) starting at high NaCl level of 50 g·L^{-1} in flow-through mode. They concluded that starting at high salinities with batch or flow-through mode produced high sulfate reduction rates of 2.8 and 3.7 g SO_4^{2-}.·L^{-1}·day^{-1}, respectively. Again, to stress the importance, the seed sludge is crucial in the success of the sulfate reducing reactor, as seen in Vallero et al. (2004) where the seed sludge from an UASB reactor treating only pulp and paper wastewater proved to be an adequate inoculum containing halotolerant SRB.

In anaerobic systems, methanogens have been found to be strongly inhibited at NaCl levels exceeding 10 g·L^{-1} (Kugelman and McCarty, 1965), while others state the IC_{50} for NaCl inhibition may be closer to 13-20 g·L^{-1} of NaCl (Lefebvre et al., 2006), which is highly dependent on the electron donor. SRBs extracted from marine sediments have been found to have a higher resistance towards salinity changes than methanogenic bacteria. Also, other salts, such as KCl, have been shown to exhibit similar inhibitory effects towards SRB and methanogenic bacteria (Zhou et al., 2011). However, in an

engineered bioreactor systems, the effects of high NaCl concentrations on sulfate reducing bacteria have rarely been investigated (Vallero et al., 2004; Vallero et al., 2005), which was one of the main drivers in conducting this research. To the author's knowledge, no one has investigated the performance of SRB treating a synthetic produced water.

H. Sulfide Removal Techniques

In practice, there are several techniques to remove sulfide from solution including stripping, chemical precipitation, chemical oxidation, and biological oxidation (Lens et al., 2002). Also, the removal of sulfide can occur in a separate stage encompassing a multi-stage treatment process, or it can occur simultaneously within another process. Sulfide stripping can be achieved through increasing turbulence in the system, or more commonly by using N_2 to strip the sulfide. In a study by Lopes et al. (2007) investigating thermophilic sulfate reduction with sucrose at pH 6, it was reported that stripping with N2 gas removed 80% of the sulfide. This technique is effective in the removal of unionized hydrogen sulfide, but it becomes ineffective at elevated pH values, around 7.5-8 or higher.

Chemical precipitation is another effective sulfide removal technique that is often used in treating acid mine drainage streams where heavy metal removal is governed by sulfide precipitation. As mentioned above in the bioreactor types section, this may be done in a one or two stage reactor system. Waters with low metals concentrations will need an external source of metals to induce sulfide precipitation including ferric or zinc salts. If the treated water is absent of metals and the purpose of treatment is to achieve high sulfate reduction rates, one should consider performing sulfide precipitation in a separate stage to minimize the competition between iron oxidizing bacteria and SRB.

Biological oxidation of sulfide is another treatment method that can achieve high sulfide removal rates. Biological oxidation of sulfides is completed by photoautotrophic or chemolithotrophic sulfur oxidizing bacteria (SOB). According to Pokorna et al. (2014), biological oxidation of sulfur can be performed by certain species of chemolithotrophic bacteria (*Thiobacillus, Sulfolobus, Thermothrix, beggiatoa and Thioxthrix*). Chemolithotrpohic SOB are mainly comprised of aerobic organisms that use oxygen as the terminal electron acceptor. The following reactions describe sulfide oxidation under aerobic conditions (Madigan et al., 2006):

$H_2S + 1/2O2 \rightarrow S^\circ + H_2O$, $\Delta G^\circ = -209.4$ kJ/reaction (Eq.1)

$S^\circ + 3/2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$, $\Delta G^\circ = -587.1$ kJ/reaction (Eq.2)

$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$, $\Delta G^\circ = -798.2$ kJ/reaction (Eq.3)

$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$, $\Delta G^\circ = -818.3$ kJ/reaction (Eq.4)

In addition, there are some chemolithotrophic SOB that can anaerobically oxidize sulfide using nitrate or nitrite as the terminal electron acceptor based on the following reactions (Cardoso et al., 2006):

$S^{2-} + 1.6NO_3^- + 1.6H^+ \rightarrow SO_4^{2-} + 0.8N_2 + 0.8H_2O$, (Eq. 5)

$\Delta G^\circ = -743.9$ kJ/reaction

$S^{2-} + 0.4NO_3^- + 2.4H^+ \rightarrow S^\circ + 0.2N_2 + 1.2H_2O$, (Eq.6)

$\Delta G^\circ = -191.0$ kJ/reaction

$S^{2-} + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^-$, (Eq.7)

$\Delta G^\circ = -501.4$ kJ/reaction

$S^{2-} + NO_3^- + 2H^+ \rightarrow S^\circ + NO_2^- + H_2O$, (Eq.8)

$\Delta G^\circ = -130.4$ kJ/reaction

The sulfur compounds most commonly used by chemolithotrophic SOB are sulfide, elemental sulfur, and thiosulfate (Tang et al., 2009). As seen in the equations above, sulfide can be directly converted to sulfate, and elemental sulfur can be oxidized to sulfate. In this experiment, the goal is to reduce sulfate and prevent calcium sulfate scaling. If biological oxidation of sulfide is to be used as a post treatment step before injection, the biological oxidation of sulfide must limit sulfate production. One way to limit the complete oxidation of sulfide to sulfate is by manipulating the dissolved oxygen in the system. It has been observed (Annachhatre et al., 2001) that at a DO levels > 0.1 $mg \cdot L^{-1}$ sulfate was main end product, while at DO levels < 0.1 $mg \cdot L^{-1}$ sulfur was the main end product. Similarly, Tang in 2009, stated that if the molar ratios of oxygen and sulfides are controlled, biological oxidation of sulfide will favor the conversion to elemental sulfur. Moreover, molar ratios of 0.53 and 1.1 for oxygen to sulfide showed incomplete oxidation of sulfide to elemental sulfur and only at ratio of 3.5 was sulfate detected (Alcántara et al., 2004). Identically for anaerobic sulfide oxidation, incomplete oxidation of sulfide to elemental sulfur will occur at low N/S ratio values of 0.32-1.0 (Pokorna et al., 2014).

Methods

A. Reactor Design

To perform the experiment, two expanded granular sludge-bed reactors with working volumes of 3.3 L were created. Reactor A was considered the control specimen and was operated with a baseline salt content of 15 $g \cdot L^{-1}$ of NaCl, while the NaCl in Reactor B was stepwise increased over time from 15 to 35 g L^{-1}. Herein, the salinity content in the reactors was based on NaCl addition, which in this case represents the total dissolved solids (TDS) content in the system. The internal diameter of each reactor was 2.25-inches, and their effective length was 50 inches, establishing a height-to-diameter ratio of around 22. A perforated plate was installed at the bottom of each reactor to uniformly distribute the flow in the expanded sludge bed. A 400-micron mesh was adhered to the plate to prevent clogging and loss of biomass during short shut-off periods for maintenance purposes. **Figure 4** shows a photo of the reactor setup.

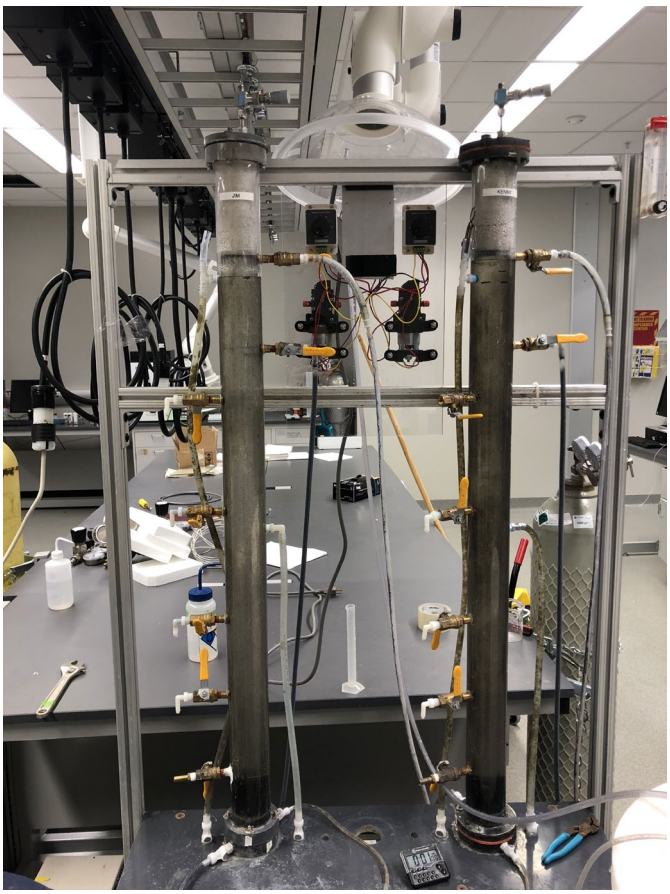

Figure 4. EGSB reactor setup.

Five sampling ports provided opportunity for sampling DNA throughout the entire water column. However, in this experiment, DNA samples were drawn only at the bottom of each reactor. The recirculation flow begins 6-inches below the effluent water level, which is then pumped to the bottom of the reactor creating a superficial upflow velocity. During startup, a superficial upflow velocity (v_{up}) of 4m·hr^{-1} was maintained to prevent biomass washout and was later increased to 5-6 m·hr^{-1} for the duration of the experiment EGSB reactors with a v_{up} of 2.5 to 5.5 m·hr^{-1} have been shown (Kato et al., 1994) to enhance mixing in the bulk liquid, and increase the contact between substrate and biomass. Therefore, higher upflow velocities have been reported to increase the mass transfer efficiency in the system. (Lens et al., 2002). The height-to-diameter ratio and

superficial upflow velocity values were selected to reduce dead spots in the reactor, to promote microbial activity, and to dilute influent concentration, specifically potentially toxic compounds commonly found in produced waters including BTEX compounds (Seghezzo et al. 1998). On the contrary, the possibility of granule shear and loss of biomass significantly increases at higher upflow velocities (Omil et al., 1996)

The influent pH in Reactor A was 7.5 ± 0.1 and the influent pH in Reactor B was 7.5 ± 0.2. See **Table A1** and **A2** in the appendix for the influent pH values for both reactors. Temperature in both reactors was maintained at 30 °C with a Fisher Scientific® Isotemp water bath. The sole electron donor used to complete sulfate reduction was propionate, suppled at a concentration of 1.94 $g \cdot L^{-1}$ as calcium propionate. Magnesium sulfate was used to supply an excess amount of sulfate, 1,000 $mg \cdot L^{-1}$. Calcium and magnesium salts were used to increase the divalent cation concentrations, which is typical in produced water compositions. In the field, the mean calcium and magnesium concentrations can range from 2,530-25,800 ppm and 530-4,300 ppm, respectively (Neff et al., 2011). The calcium and magnesium concentrations in the synthetic produced water used in this study did not fall within these ranges, but as seen below in the well chemistry data, calcium and magnesium can be 500 and 0 ppm, respectively. Other salts added to simulate a synthetic produced water (Shardi et al., 2013) were the following final compounds in $g \cdot L^{-1}$: 0.1 $CaCl_2 \cdot 2H_2O$; 0.333 KCl; 0.092 $MgCl_2 \cdot 6H_2O$; and 0.113 $NaHCO_3$. Nitrogen and phosphorous were supplemented following Redfield's ratio of 106 C: 16N:1P using 0.432 $g \cdot L^{-1}$ of NH_4Cl and 0.031 $g \cdot L^{-1}$ of KH_2PO_4. In addition, 0.5 mL of a trace metal solution was added per liter of influent water. The trace metal solution was comprised of 0.5 $g \cdot L^{-1}$ of the following chemical compounds: H_3BO_3; $ZnCl_2$; $(NH_4)_6Mo_7O_{24}.4H_2O$; $NiCl.6H_2O$;

$AlCl_3.6H_2O$; $CoCl_2.6H_2O$; $CuSO_4.5H_2O$; $NaSeO_3.5H_2O$; and $MnCl_2.4H_2O$. In addition, 0.5 mL of a 2.7 $g \cdot L^{-1}$ FeSO4 stock solution was added per liter of influent water (Dries et al., 1998).

B. Inoculum

The seed granules used to inoculate the reactors were collected from a full-scale UASB in Cedar Rapids, Iowa wastewater treatment plant. The plant has high loads of industrial wastewaters, specifically 10 MGD from a paper mill plant. The granules were rinsed and washed on a 400-micron steel mesh to remove silt and sand particles. Approximately, 50 mL of biomass were added to each reactor before startup. The VSS concentrations in Reactors A and B were 9,950 $mg \cdot L^{-1}$ and 7,040 $mg \cdot L^{-1}$, respectively.

C. Procedure to Acclimate Granules to salinity:

The NaCl levels throughout the experiment for the EGSB reactors can be seen below in **Table 5**. The startup NaCl level for each reactor was 10 $g \cdot L^{-1}$ (1% salinity), which was then increased to 15 $g \cdot L^{-1}$ in both reactors. To assess the effects of increasing the NaCl content on sulfate reduction, Reactor A was established as the control, and the NaCl content in Reactor B was stepwise increased to 35 g L^{-1}. In Reactor B, two schemes were investigated to determine the overall effect of NaCl on sulfate reduction. Scheme 1 included a salinity stress test of rapidly increasing the NaCl content from 15 to 40 $g \cdot L^{-1}$ of NaCl and Scheme 2 encompassed gradually increasing the NaCl in 0.5 $g \cdot L^{-1}$ increments.

Table 5. NaCl concentrations in Reactor A and Reactor B.

Reactor	Description	Time (Days)	NaCl ($g \cdot L^{-1}$)
Reactor A			
	Starting Condition	0-41	10
	Base Condition	42-92	15
	Salinity Stress Test	93-109	40
	Base Condition	110-177	15
Reactor B			
	Starting Condition	0-41	10
	Baseline Condition	42-81	15
	Scheme 1 (Stress Test)	82-103	40
	Baseline Condition	104-114	15
	Scheme 2 (Slow Acclimation)	115-136	20
		137-156	25
		157-177	30
		178-185	35

D. Chemical Analysis

Chemical analysis for the experiment included analyzing propionate and acetate using a gas chromatograph. The gas chromatograph was an Agilent Model CP9212 (VF-Waxms) with a 30 m long and 320 µm diameter capillary column with a 0.25 µm film thickness. The flow rate of the carrier gas (helium) was 1.2 $mL \cdot min^{-1}$, and the column pressure was 7.8 psi. The column temperature was 80°C for 5 minutes and then was ramped up at a rate of $3°C \cdot min^{-1}$ until reaching 120°C.The sampling port temperature was 240°C and the detector was a mass spectrometer with MS source temperature and MS guage temperature of 230°C and 130°C, respectively, while the transfer line was at 220°C.

Samples collected for volatile fatty acid (VFA) analysis were filtered through a 0.45-micron nylon filters from Fisher Scientific and then were acidified with a 0.02M oxalic acid solution and stored at -4°C.The minimum detection limit of acetate was determined to be 0.817 $mg \cdot L^{-1}$ acetate by following the methods described by Childress et al. (1999). Refer to **Table A3** in the Appendix.

Sulfate was analyzed using an ion chromatograph. The ion chromatograph was a Dionex Model ICS-2000 with a Dionex Ionpac AS18 column and a Dionex Ionpac AG18 guard column with an eluent concentration of 30 mM KOH at flow rate of 1 $mL \cdot min^{-1}$. The ions were suppressed at conductivity of 75 mAmps. To prevent pressure buildup due to the scaling in the column, influent and effluent samples were diluted with distilled water at ratios of 1:200 and 1:100, respectively. Serial dilutions were performed to reach the desired dilution criteria, and after the first serial dilution factor of 10, two drops 0.4 M zinc acetate solution were added to precipitate the sulfide. Sulfate lost to co-precipitation was negligible as the difference in sulfate analysis results with and without zinc acetate were < 5%. Samples collected for sulfate analysis were filtered with 0.45-micron nyolon filters from Fisher Scientific and stored at -4°C.

A one-tailed Student t-test with a 95% confidence interval (α=0.05) was conducted using Microsoft Excel to test the statistical difference between the sulfate removals in Reactor A and Reactor B

Total dissolved sulfide analysis was performed following Standard Method 4500-S^{-2} Part F. Iodometric Method (APHA et al., 2005). Samples collected from the reactor effluent were analyzed right away to prevent loss of H_2S. If solids were present, collected sample was filtered through a 0.45-micron filter. The iodine solution and thiosulfate

solution used in the titration procedure were normalized every week to reduce error in the results. pH was measured using an Accument AB2000 pH meter from Fisher Scientific. Since the influent pH of both reactors was approximately 7.5, the equilibrium concentrations of H_2S and HS^- in the system can be determine by the equilibrium reaction shown below if the total sulfide concentration in solution is known.

$$H_2S \leftrightharpoons HS^- + H^+$$

In saline waters, an activity correction must be applied to the equilibrium constant (k1) for the equilibrium reaction specified above. Shown in **Table 6** is a list of corrected first dissociation constants for hydrogen sulfide over a range of salinity levels at 30°C.

Table 6. First dissociation constants of hydrogen sulfide at various salinities (APHA, 2005).

pK1 at a Given Salinity Content					
10 g·L^{-1}	15 g·L^{-1}	20 g·L^{-1}	25 g·L^{-1}	30 g·L^{-1}	35 g·L^{-1}
6.63	6.60	6.57	6.56	6.55	6.54

Assuming that only dissolved sulfides were included in the total dissolved sulfide concentration, the dissolved undissociated sulfide can be calculated from the total sulfide in the system, the system pH, and the adjusted dissociation constants. This is represented by the equation below (APHA, 2005).

$$\alpha_{H2S} = [H_2S] \cdot [\text{Total Sulfide Concentration}]^{-1} = 1 \cdot (10^{pH} - 10^{k1} + 1)^{-1}$$

E. PHREEQC Modeling

To determine the relevant application of biologically reducing sulfate levels in produced waters, the scaling potentials of selected sulfate salts, in produced waters representative of those commonly encountered in the Minnelusa oil formation, were

estimated using a geochemistry software program called PHREEQC. PHREEQC version 3.4 is a free public domain program that has the capability of performing a wide range of geochemistry calculations (Parkhurst and Appelo 2013).

Table 7 shows the produced water quality characteristics for the three oil producing wells used in the PHREEQC modeling effort described herein. The wells are located in the Minnelusa-B formation in Wyoming. The observed pH values for Wells 1, 2, and 3 were 7.20, 7.16, and 7.60, respectively. In addition, the depths of the wells were 7,937 ft, 7,569 ft, and 7,320 ft. **Table 8.** shows the composition of a groundwater source used as an injection water in the Bracken field well (Well #3).

Table 7. Produced water composition for Well 1: Simpson North Oil Field; Well 2: Pownall Ranch Oil Field; and Well 3: Bracken Oil Field (Wyoming Oil & Gas Conservation Commission, 2013)

Well 1		Well 2		Well 3	
Constituent	**Concentration (mg/L)**	**Constituent**	**Concentration (mg/L)**	**Constituent**	**Concentration (mg/L)**
Na^+	10,536.00	Na^+	8,900	Na^+	41,452
K^+	0	K^+	320	K^+	0
$Ca2^+$	500	$Ca2^+$	1,900	$Ca2^+$	1,520
Mg^{2+}	41.3	Mg^{2+}	550	Mg^{2+}	0
SO_4^{2-}	4,664.40	SO_4^{2-}	1,220	SO_4^{2-}	1,622
Cl^-	12,747	Cl^-	19,500	Cl^-	66,770
HCO_3^-	1,830	HCO_3^-	268	HCO_3^-	708
Fe^{2+}	0	Fe^{2+}	0	Fe^{2+}	3.60
TDS	30,319	TDS	32,500	Estimated TDS	78,334
Ionic Strength	0.55	Ionic Strength	0.64	Ionic Strength	1.96

Table 8. Water quality composition of a groundwater used as injection water in Well 3.

Constituent	Concentration (mg/L)
Na^+	384
K^+	0
Ca^{2+}	440
Mg^{2+}	122
SO_4^{2-}	913
Cl^-	486
HCO_3^-	976
Fe^{2+}	1.60
Estimated TDS	2,569
Ionic Strength	0.06

Calcium sulfate has three predominant solid forms: gypsum, hemihydrate, and anhydrite; however, the scaling potential was conducted only for gypsum and anhydrite as these scales are the most common in the oil and gas industry. The general equations for the dissolution of anhydrite ($CaSO_4$) and gypsum ($CaSO_4*2H_2O$) are shown below

$$CaSO_4 \leftrightarrow Ca^{2+}\ SO_4^{-2} \qquad \text{(Eq. 9)}$$

$$CaSO_4 \cdot 2H_2O \leftrightarrow Ca_2+ + SO_4^{2}- + 2H_2O \qquad \text{(Eq.10)}$$

In addition, the solubility product is written from the dissolution equation, which is shown below.

$$Ksp = \{Ca^{2+}\} * \{SO_4^{-2}\} \qquad \text{(Eq. 11)}$$

Even though the two minerals have the same dissolution products, their equilibrium constants differ. The difference in the solubility between gypsum and anhydrite is in the

molecular structure. The saturation index (SI) is calculated as shown in the equation below.

$$SI = \log\left(\frac{\{\text{Ion Activity Product}\}}{\text{Ksp}}\right) \qquad \text{(Eq. 12)}$$

If SI > 0, the solution is oversaturated at current levels of calcium and sulfate. Once this occurs, calcium sulfate will spontaneously precipitate out of solution until equilibrium is reached, at SI=0. When SI is < 0 the, the solution will be undersaturated for that phase and will not form $CaSO_4$ precipitates, but will dissolve until SI = 0.

In PHREEQC, there are several databases that can be used to model water-based chemical reactions. In this paper, databases are defined as the ASCII files that contain thermodynamic data that can be modified or changed by the user. The databases selected were the phreeqc.dat and pitzer.dat databases. The differences between databases is the inventory of chemical compounds, the thermodynamic data, and the activity correction equations. For produced water conditions with relatively high TDS and ionic strengths > 1M, the pitzer.dat would typically be selected to account for the high activity in the water. With low to moderate TDS waters the phreeqc.dat is acceptable where the phreeqc database calculates the activity corrections by using the Extended Debye-Hückel equation. Horbrand et al. (2018) reported that the ionic strength limit for the activity model calculated from the Extended Debye-Hückel equation is between 0.1-3 M. In this paper, the phreeqc.dat was selected for all simulations. The pitzer database, while best for high ionic solutions, contains only a limited number of mineral phases in the inventory list. The phreeqc.dat may not be the best suited database for high ionic strength waters, but was selected in order to model the formation of ZnS precipitates.

Five scenarios were simulated (modeled) using PHREEQC. Simulation I was performed to investigate how temperature and pressure changes impact gypsum and anhydrite scaling potential. Simulations II-IV modeled the scenario of reinjecting each well's produced water back in the reservoir via one injection well. The focus of each simulation was to track the scaling potential for gypsum and anhydrite with pressure and temperature changes over the length of the injection wells. Simulations II-IV model theoretical water flooding applications of injection water near an oil producing well to enhance oil production. Refer to **Figure 5** (Caudle, 2018) for an illustration of how water flooding works.

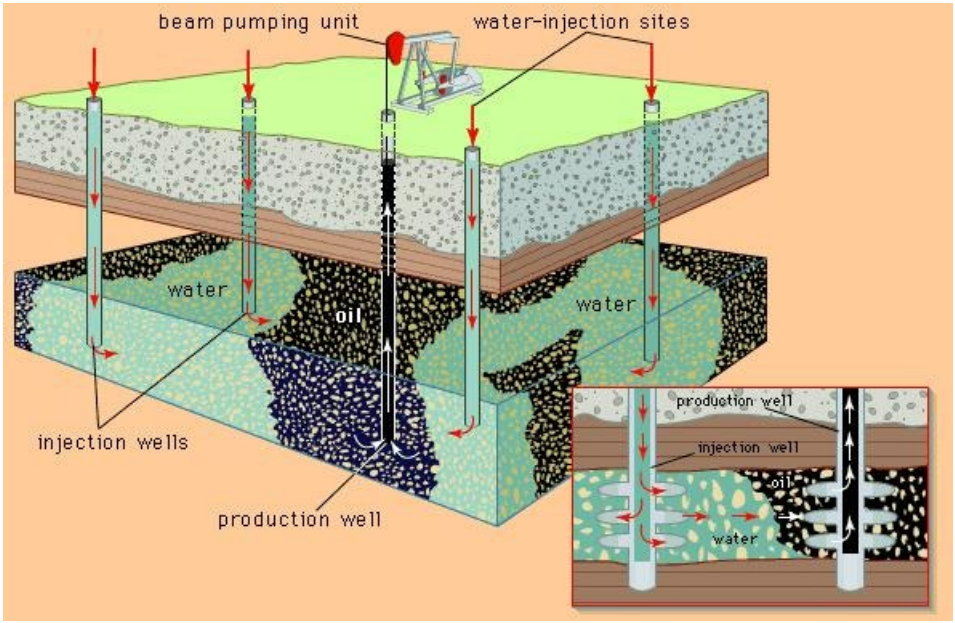

Figure 5. Schematic of water flooding application (Caudle, 2018).

The temperature gradient from the surface to the bottom of the wells was assumed to be 0.01 °F / ft. of well depth. With an average well depth of around 7,600 ft. and a temperature gradient of 0.01°F / ft. of well depth, the temperature range from the surface to the bottom of each well included temperatures of 25 to 70°C. Since all three wells were

similar in depth and were located in the same formation, the pressure changes in all three wells were assumed to be the same. The pressure gradient was assumed to follow a linear trend, and pressures were assumed to increase from 1 to 300 atm. The REACTION_PRESSURE title block was used to simulate this pressure range from the top of the well to the bottom of the well. In simulation V, a sensitivity analysis for predicting oversaturated conditions for gypsum at various Ca/SO_4^{2-} ratios and NaCl levels was conducted.

Results

Two reactors were inoculated with UASB granules collected from a municipal wastewater treatment plant treating pulp and paper wastewater. Reactor A was set as the control of the experiment with a baseline salinity content of 15 $g \cdot L^{-1}$ while the salinity in Reactor B was gradually increased to 35 $g \cdot L^{-1}$ of NaCl. A salinity stress test was performed in each reactor to observe the effects of rapidly increasing the salinity from 15 to 45 $g \cdot L^{-1}$ of NaCl. The overall performance of both EGSB reactors throughout the duration of the experiment was based on the dissolved sulfide concentration, sulfate removal (reduction), and the VFA (propionate and acetate) levels.

A. Reactor A Startup Performance

The startup performance, in regard to sulfate removal, of Reactors A and B can be seen below **Figure 6** and **Figure 7,** respectively. During initial startup, the salinity content in both reactors was at 10 $g \cdot L^{-1}$ of NaCl. In Reactor A, the seed granules were quick to adapt to the new system, as a sulfate removal of 90% was achieved on day 15 (Fig. 6). After day 19, sulfate removal increased to > 90%. Likewise, the seed sludge in Reactor B was quick to adapt to the low salinity environment, and a sulfate removal of 86.0% was observed on day 15.

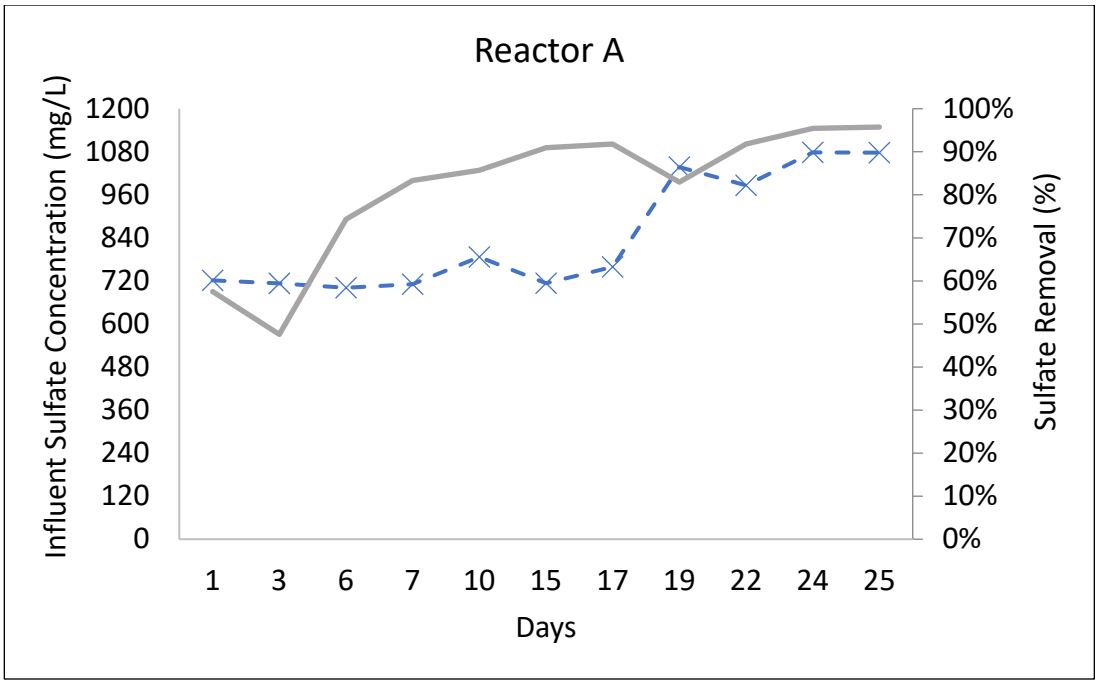

Figure 6. Startup performance for Reactor A.: (—) Sulfate reduction; (X) Influent sulfate concentration.

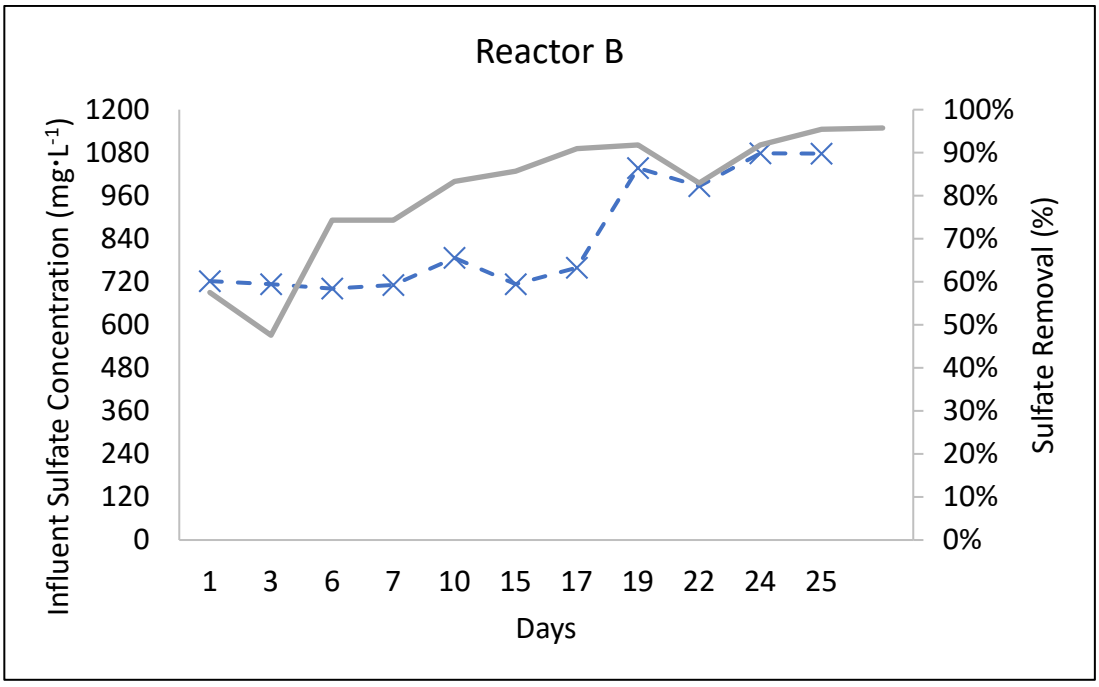

Figure 7. Startup performance for Reactor B.: (—) Sulfate reduction; (X) Influent sulfate concentration.

B. Sulfide Production & Sulfate Removal Performance

In Reactor A, the baseline salinity level of 15 g·L^{-1} of NaCl was maintained for 117 days (Fig. 8). During this time period, with the baseline salinity level as seen in **Figure 8a** and **Figure 8b**, the average total sulfide concentration in the effluent was 321 ± 12.3 mg·L^{-1} of S^{2-}, and the sulfate removal was > 90.0%. For comparing Reactor A to Reactor B, these values were considered the baseline performance levels. On day 93, the salinity content was rapidly increased from 15 to 40 g·L^{-1} of NaCl. This salinity level was maintained for 16 days. On day 107 (after 14 days at the higher salinity), it was clear that the rapid increase in salinity was impacting the system performance as the total sulfide concentration in the effluent dropped to 242 mg·L^{-1} of S^{-2}. A few days after observing this decline in system performance (day 110), the system salinity was reduced back to the baseline salinity. Remarkably, the system's total sulfide concentration rebounded back to 311 mg·L^{-1} of S^{2-} only three days after reducing the salinity back to 15 g·L^{-1}. This indicated that the impact from the high salinity content was reversible and that the SRBs were quick to recover from rapid changes in salinity. See **Table A4** in the appendix for additional sulfate removal data in Reactor A.

Reactor B included two schemes to acclimate the biomass to salinity, as seen in **Figure 9**. Refer to **Table A5** in the appendix for more sulfate removal data. At the beginning of Scheme 1, a salinity content of 15 g·L^{-1} was maintained for 39 days. Statistically comparing the sulfate removals recorded during this time for Reactor A and Reactor B, using a one-tailed Student t-test, gave a p-value of 0.33. The statistical analysis showed that the sulfate removal in Reactor B at a salinity content of 15 g·L^{-1} was

statistically similar to Reactor A's baseline sulfate removal before the salinity stress test took place. See **Table A6** in the appendix for information on the Student t-test analysis.

Scheme 1 for Reactor B included performing a salinity stress test, as was done in Reactor A, by rapidly increasing the salinity content from 15 to 40 $g \cdot L^{-1}$. In Scheme 2, the salinity content was stepwise increased to 35 $g \cdot L^{-1}$ of NaCl. During Scheme 1 (**Figure 9a)**, the total sulfide concentration for the first 10 days was relatively constant at approximately 260 $mg \cdot L^{-1}$ of S^{2-} . The percent difference between the total sulfide concentrations on day 82 and day 95 was only 4.5%. However, this trend did not continue and the total sulfide concentration decreased to 90 $mg \cdot L^{-1}$ of S^{2-} on day 103. In **Figure 9b**, the sulfate removal efficiency also exhibited a similar response, but the sulfate removal performance decreased immediately from 92.0% to 84.0% after the first day of increasing the salinity to 40g/L of NaCl. Once the sulfate removal efficiency dropped to 84.0%, the removal efficiency was relatively constant until the sulfate removal efficiency decreased again on day 95, and then continued to decrease to the lowest sulfate removal efficiency recorded for Reactor B of 32.0% on day 103 The salinity stress test lasted 21 days and a 61% decrease in the sulfate removal performance was observed from the beginning (day 82) to the end (day 103) of the salinity stress test. Rapidly acclimating the biomass to high salinity was an inadequate method to adapt the bacterial community to salinity, which is why the salinity was reduced back to baseline salinity level to prepare the biomass for slowly acclimating the biomass to salinity.

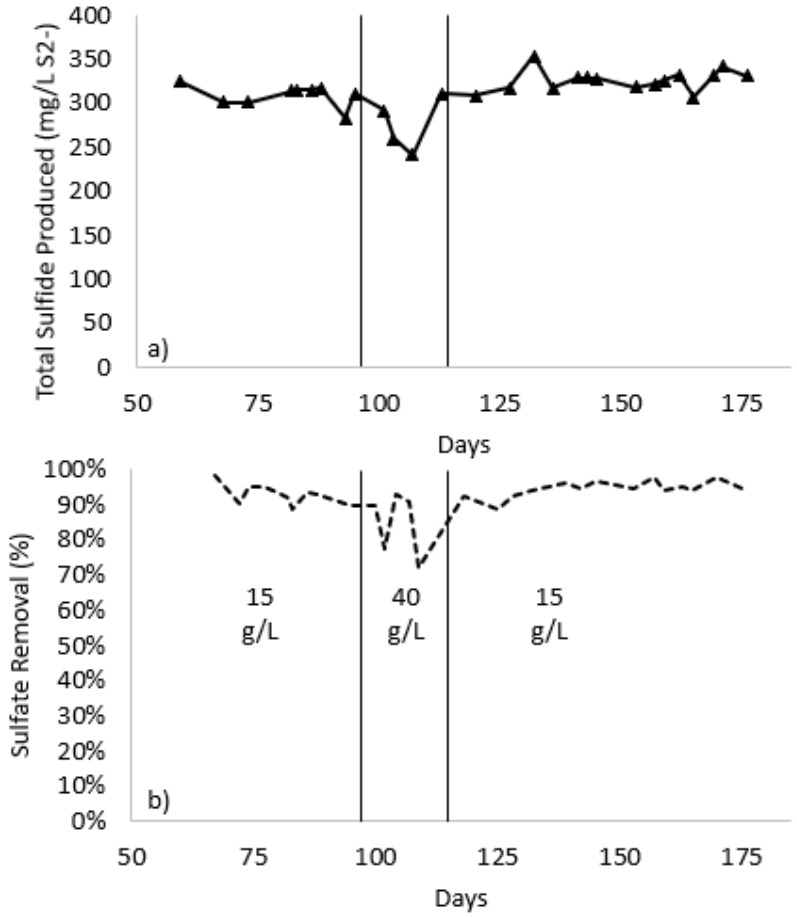

Figure 8. Reactor A: a) Total sulfide concentration; b) Sulfate removal efficiency

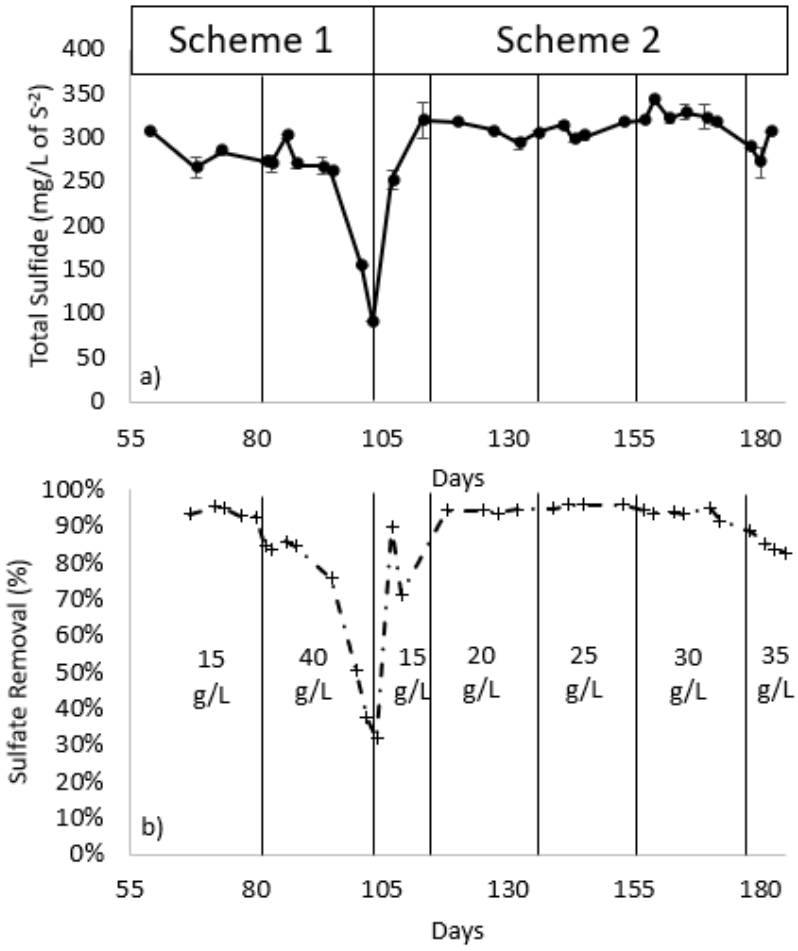

Figure 9. Reactor B: a) Total sulfide concentration; b) Sulfate removal efficiency

Before Scheme 2 started, a salinity level of 15 g·L^{-1} was maintained for 10 days to allow the biomass to recover from the salinity shock performed in Scheme 1. The sulfate reduction performance and the sulfide levels were able to rebound 4 days after the salinity shock, achieving a 90.0% sulfate removal efficiency and producing 252 mg·L^{-1} S^{2-} on day 107. Instead of rapidly increasing the salinity, Scheme 2 encompassed increasing NaCl in 5.0 g·L^{-1} increments from 20 to 35 g·L^{-1}. During the time period with a NaCl content of 20-30 g·L^{-1}, the average sulfate removal efficiency was 94.0 ± 1.2%, which was statistically similar to Reactor A's baseline sulfate removal efficiency (p-value =0.46). See

Table A7 in the appendix for information on the Student t-test. In addition, Reactor B achieved the highest sulfate removal efficiency of 96.0% during the experiment at a NaCl level of 25 g·L^{-1}. In **Figure 9a** for NaCl level of 20-30 g·L^{-1}, the total sulfide levels appeared to decrease for a few days after each incremental increase of NaCl, but were quick to increase to around 300 mg·L^{-1} of S^{2-}. After the incremental salinity increase to 35 g·L^{-1} of NaCl, the sulfide concentration decreased to 280 mg·L^{-1}, and the sulfate removal efficiency decreased to 84%. Based on the total results recorded after stepwise increasing salinity from 20-35 g·L^{-1} of NaCl, the sulfate removal efficiencies were statistically similar (p-value of 0.052) to the baseline sulfate removal efficiencies in Reactor A at salinity content of 15 g·L^{-1} of NaCl. See **Table A8** in the appendix for more information on the Student t-test.

In Scheme 2 at a salinity content of 25 g·L^{-1} of NaCl (Fig 8b), the average total sulfide level was 307.4 ± 9.0 mg·L^{-1} of S^{2-}. At this time period in Reactor B with an average pH value of 7.9 ± 0.2, the average undissociated sulfide concentration was calculated as 13.14 mg·L^{-1} of S^{2-}, indicating the dissolved hydrogen sulfide concentration posed little toxicity risk to the SRB since IC_{50} for hydrogen sulfide has been reported to be 30-250 mg·L^{-1} (Pokorna et al., 2014).

C. Propionate degradation results

A COD/SO_4 ratio of 1.5-2.3 was maintained throughout the duration of the experiment with addition of propionate. See **Table A9** and **A10** in the appendix for the COD/SO_4 results. In the biological sulfate reduction process with propionate used as sole electron donor, SRB can completely oxidize propionate to carbon dioxide or can partially oxidize propionate to acetate and carbon dioxide. The dominant metabolic pathways is

dependent on the SRB strains present in the bacterial population. The VFA results included the analysis of propionate and acetate (Fig. 10 and Fig. 11). Propionate was selected as the electron donor, and acetate was monitored to see if incomplete oxidation of propionate to acetate was occurring. After the salinity shock, the propionate removal efficiencies in Reactor A and Reactor B were similar with an approximate removal efficiency of 70% (Fig 10a. and Fig. 11a). Performing a one-tailed Student t-test with a 95% confidence interval for the propionate removal efficiencies recorded during these days, a p-value of 0.11 was determined. See **Table A11** in the appendix for information on the Student t-test.

One known disadvantage of using organic acids as the electron donor is the high COD in the effluent. In **Figure 10a**, the effluent acetate concentration in Reactor A was relatively low during the salinity spike. Otherwise the effluent acetate concentration was relatively high with a maximum concentration of 621 mg of COD·L^{-1} observed on day 145. In Reactor B (Fig. 11b), from day 107 until the end of the experiment, the acetate effluent concentration was lower than in Reactor A, albeit the propionate removal efficiency of 70% remained similar between the two reactors. Based on the dilution factors used in the influent samples for VFA analysis, the influent acetate levels were considered to be non-detects with a minimum detection limit of 0.817 mg of acetate ·L^{-1}. See appendix for information about determining the minimum detection limit for acetate.

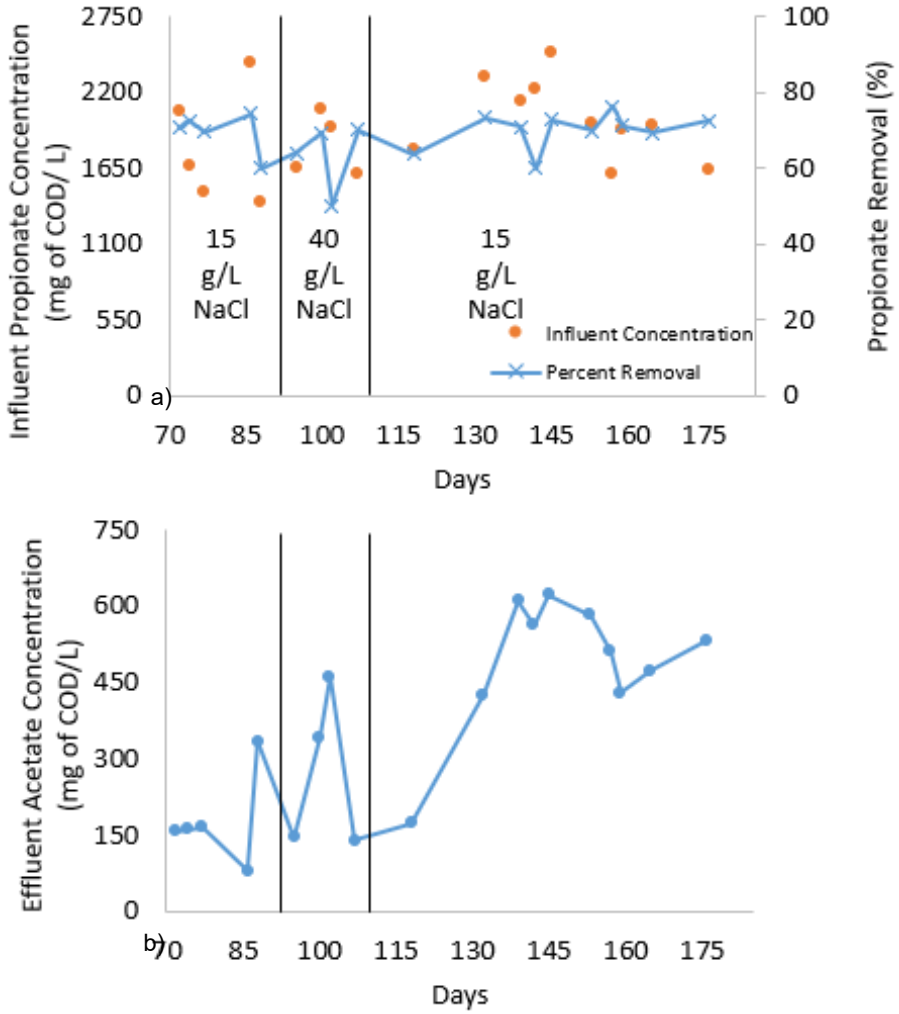

Figure 10. Reactor A: a) Propionate biological degradation: (—) Propionate removal efficiency; (●) Influent propionate concentration; b) Acetate concentration in effluent.

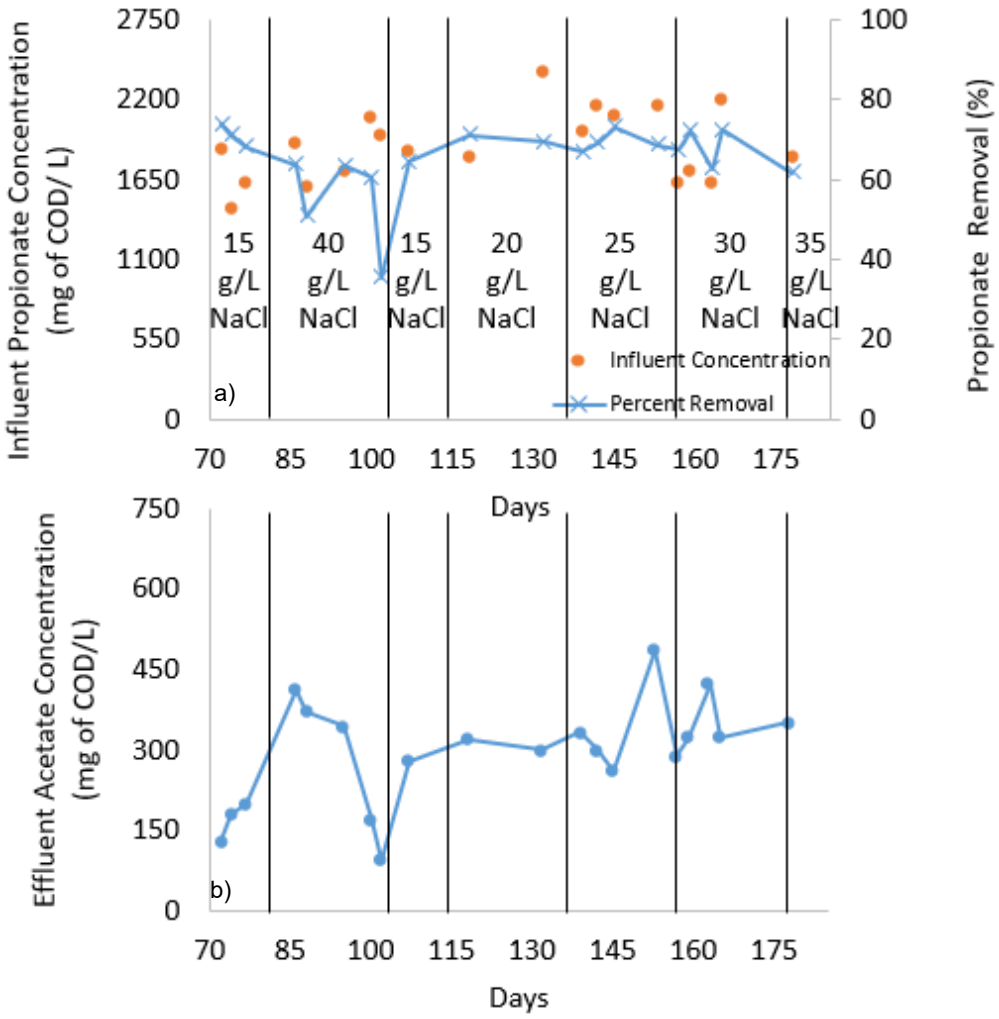

Figure 11. Reactor B: a) Propionate biological degradation: (—) Propionate removal efficiency; (●) Influent sulfate concentration; b) Acetate concentration in effluent.

D. PHREEQC MODELING

Three produced water compositions from three different oil producing wells in Wyoming's Minnelusa formation (Table 7) were selected for modeling using PHREEQC. In this type of formation, $CaSO_4$ scale is a common issue in oil production, which often results in temporary shutdown periods in order to remove the scale buildup in the system. To determine the relevane of using a sulfate reducing bioreactor in the oil and gas industry, the scaling potentials of anhydrite and gypsum were monitored in each

simulation. The temperature gradient used in the PHREEQC model was obtained directly from the oil log data sheets retrieved from the Wyoming Oil & Gas Conservation Commission (2013). The pressure change as a function of well depth was assumed to follow a linear relationship from 1 atm at the surface to 300 atm at the bottom.

i. Simulation I – Gypsum and anhydrite scaling with pressure and temperature changes.

Well 3's produced water composition was used in this simulation to show the stability of gypsum and anhydrite. The ionic strength of Well 3's produced water (1.96) was the highest out the three wells, and the sulfate and calcium levels were 1,622 $mg \cdot L^{-1}$ and 1,520 $mg \cdot L^{-1}$, respectively. **Figure 11** shows the scaling potential of gypsum and anhydrite at various temperatures and pressures using Well 3's produced water composition. The phase transition temperature between gypsum and anhydrite occurs when the two lines intersect, as shown in **Figure 12**. In terms of solubility, anhydrite has a higher solubility than gypsum at temperatures below the transition temperature, while gypsum is more soluble at temperatures greater than the transition temperature. The transition temperature of gypsum to anhydrite appeared to increase with increasing pressure as seen by comparing 1 atm and 300 atm, where the transition temperatures were approximately 48 °C, and 52.5 °C, respectively. In addition, at higher pressures, the scaling potential of both sulfate salts decreased with increasing pressure. With this in mind and incorporating the time retention time in the oil separator, it was assumed that the produced waters were in equilibrium with gypsum instead of anhydrite. Therefore, the EQUILIBIRUM_PHASES title block was set to gypsum for Simulation II-IV.

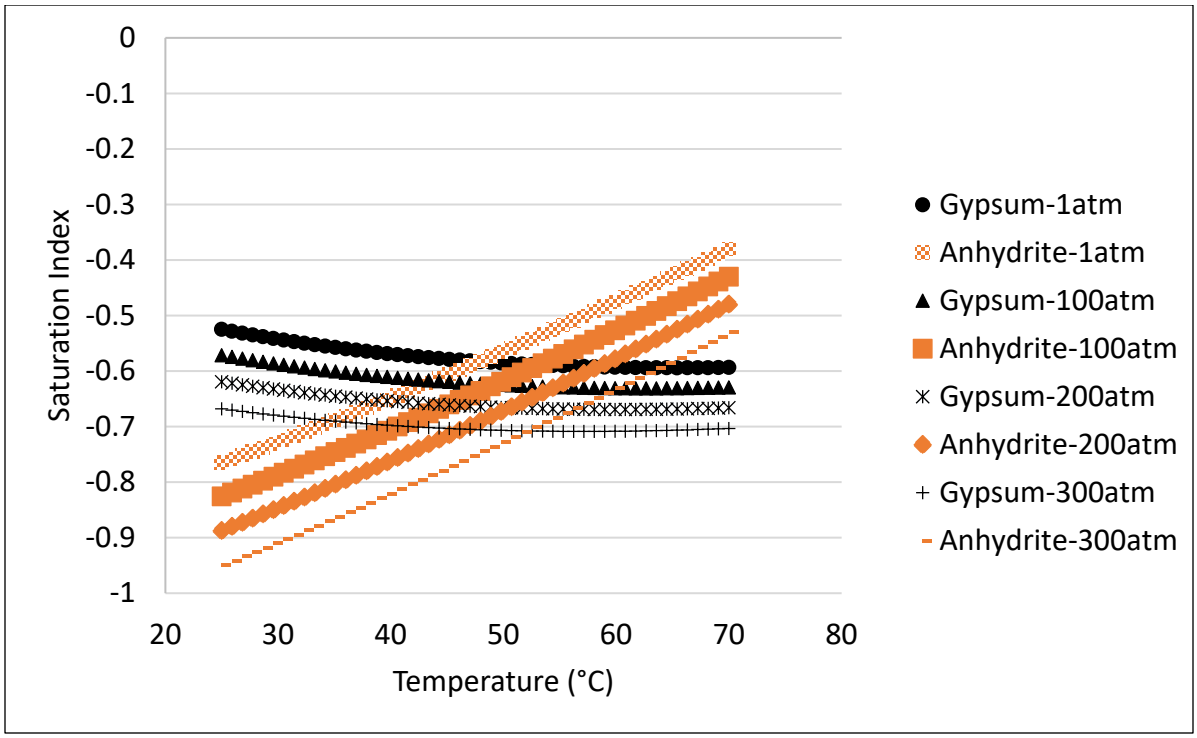

Figure 12. Gypsum and Anhydrite scaling potential with varying temperature and pressure using Well 3's produced water composition.

ii. Simulation II – Gypsum and anhydrite scaling potential for all three wells during Injection**.**

The scaling potential of the synthetic produced water was modeled at 1 atm over a temperature range of 25-70°C. Thermodynamically, the scaling potential of a selected mineral is related to the saturation index (SI). If SI is > 0, spontaneous precipitation will occur, and if the SI < 0, dissolution will occur. The highest scaling potential for anhydrite was observed at 70°C with an SI value of around -0.75 (Fig. 12a). For gypsum, the highest scaling potential was observed at 30°C with an SI value of -0.81. At normal reactor operation conditions of 1 atm and 30°C, the scaling potential was low for the synthetic produced wastewater, indicating the spontaneous scaling potential for anhydrite or gypsum was not thermodynamically favorable. **Figure 13b** and **Figure 13c** show the

scaling potential of gypsum and anhydrite for each produced water along the depth of the well, respectively. The REACTION_PRESSURE title block was used to simulate pressure range between 1-300 atm. Although the saturation indices are negative, there still may be scale formation due to heterogeneous precipitation, which will be discussed in greater detail later in the paper. For gypsum and anhydrite, Well 1's produced water had the highest scaling potential, while Well 3's produced water had the lowest scaling potential. Comparing the scaling potential of anhydrite and gypsum in each well, the highest saturation index value of -0.08 was observed at surface conditions for gypsum. Based on the produced water compositions and the temperature and pressure changes, gypsum was more likely to form than anhydrite. This is in agreement with Moghadasi et al. (2006) who reported that anhydrite will be the dominant phase at temperatures exceeding 120 °C. The kinetics of the formation of gypsum and anhydrite were not accounted for in this simulation or in the sequential simulations described below.

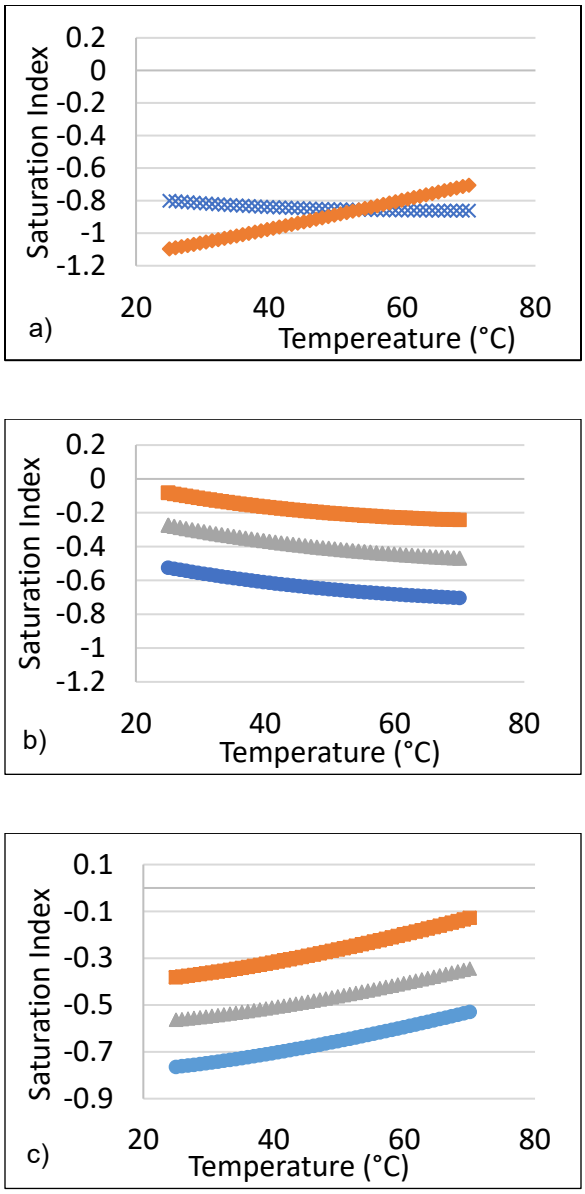

Figure 13. a) $CaSO_4$ scaling potential for synthetic produced water after injection: (■) Gypsum scaling potential along average well depth; (♦) Anhydrite Scaling potential along average well depth; b) Gypsum scaling potential after injection: (■) Well 1; (▲) Well 2; (✹) Well 3; c) Anhydrite scaling potential along the depth of injection well: (■) Well 1; (▲) Well 2; (✹) Well 3.

iii. Simulation III – Gypsum scaling potential for all three wells after theoretical treatment with sulfate reducing bacteria.

In simulation III, PHREEQC was used to estimate the scaling potential of gypsum after theoretically treating the produced water with a sulfate reducing EGSB reactor. It

was assumed that sulfate removal would be at least a 90% for each produced water. The pH was kept constant before and after the sulfate reduction. Since the rate of formation of sulfate salts is nearly pH independent, deviations from this assumption would not greatly change the results. Sulfide was accounted for, and was theoretically removed by adding zinc and forming ZnS precipitates. PHREEQC accounts only for homogenous precipitation. Having past knowledge that Well 3 has had oil production issues from $CaSO_4$ scale, it was assumed a saturation index value > -0.6 may induce heterogeneous precipitation. The green shaded saturation index values indicate minimal gypsum scaling potential while the red shaded values indicate gypsum formation may occur. Looking at the saturation indices, Well 1 had the highest scaling potential for gypsum. A sulfate removal efficiency of ≥ 70% would be required to ensure that gypsum will not heterogeneously form. Based on the criteria for heterogeneous scaling potential for gypsum, the sulfate removal performance in the EGSB reactor would need to be ≥ 55% in treating Well 2's produced water. For Well 3, ≤ 50% sulfate removal performance level would be sufficient to reduce the scaling potential in the system.

Table 9. Gypsum scaling potential after biological treatment. Green shaded area = heterogeneous precipitation of gypsum is not likely to occur; Red shaded area = heterogeneous precipitation of gypsum may occur.

Percent Removal of Sulfate	Well 1	Well 2	Well 3
90%	-1.01	-1.26	-1.52
85%	-0.89	-1.09	-1.34
80%	-0.77	-0.96	-1.22
75%	-0.68	-0.87	-1.12
70%	-0.60	-0.79	-1.04
65%	-0.54	-0.72	-0.98
60%	-0.48	-0.67	-0.92
55%	-0.44	-0.62	-0.87
50%	-0.39	-0.57	-0.82
Raw Produced Water Injection (0%)	-0.08	-0.27	-0.53

iv. Simulation IV – Blending scenario with local groundwater source.

Blending produced water with freshwater is a common practice in the oil and gas industry, one designed to minimize the amount of freshwater used during operation. In **Figure 14**, produced water from Well 3 and the local groundwater source identified in **Table 8** were blended together at different ratios to observe the gypsum scaling potential during injection. **Table 10** shows the maximum recorded saturation indices for each blending ratio. Based on the estimated minimum saturation index value of -0.6 specified above to induce heterogeneous scaling, all blending ratios decreased the scaling potential of gypsum. However, blending these two waters together substantially increased the scaling potential of calcite well into supersaturation conditions. Blending may seem advantageous in many scenarios but one should first consider the water chemistry of both solutions before mixing.

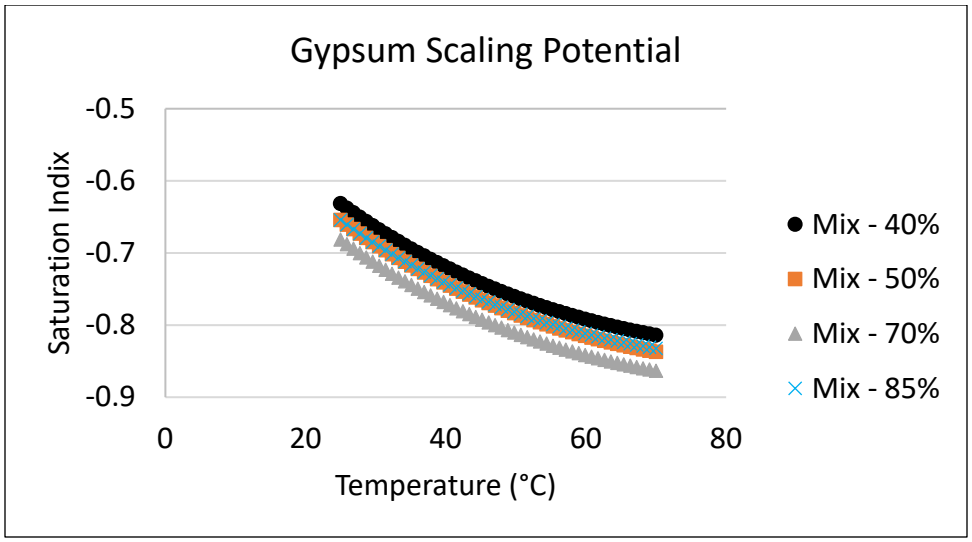

Figure 14. Gypsum scaling potential after blending with Well 3 produced water and Fox Hill's groundwater.

Table 10. Gypsum and calcite scaling potentials after blending groundwater (GW) and Well 3 produced water (PW) at 25°C and 1 atm.

SI (Gypsum)	SI (Calcite)	Blending Ratio (GW: PW)
-0.53	0.48	0%
-0.63	0.72	40%
-0.65	0.79	50%
-0.68	0.93	70%
-0.65	1.07	85%

v. Simulation V – Sensitivity analysis for homogenous precipitation of gypsum.

A sensitivity analysis was conducted to determine the conditions required in PHREEQC to spontaneously precipitate gypsum (SI>0). To determine these conditions,

different Ca/SO_4^{2-} ratios at specific NaCl levels were used to evaluate the scaling potential of gypsum. The results were then collected and displayed in **Figure 15**. The sulfate concentration was kept constant at 1,620 $mg \cdot L^{-1}$ while Ca^{2+}, Na^{+}, and Cl^{-} were adjusted to the desired Ca/SO_4^{-2} ratios and NaCl levels. Other constituents in the water were similar to the synthetic produced water characteristics. The Ca/SO_4^{2-} ratios were directly proportional to the gypsum scaling potential. As the NaCl levels increased, the scaling potential of gypsum decreased. Overall, oversaturation conditions in the formation of gypsum were reached when the Ca/SO_4 ratios were 1.5-2.5 for waters with a NaCl level of 15-60 $g \cdot L^{-1}$. As previously mentioned, this indicates homogenous precipitation will occur, but does not necessarily reflect the conditions to cause heterogeneous precipitation.

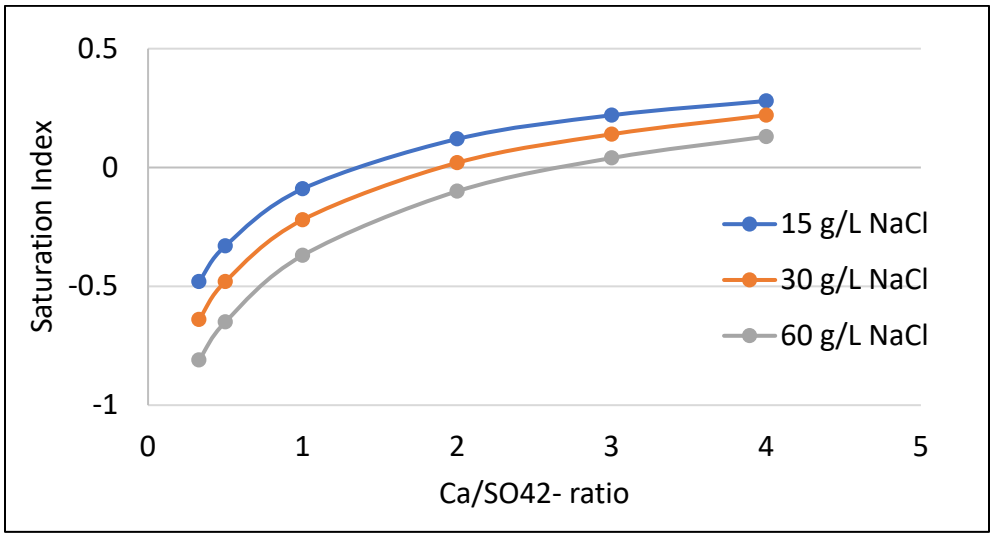

Figure 15. Sensitivity analysis for gypsum scaling potential at 25°C and 1 atm.

Discussion

A. Startup

Unadapted UASB granules were successfully acclimated to a synthetic produced water at low to moderate salinity levels (20-35 $g \cdot L^{-1}$ of NaCl). The seed granules from a UASB reactor at a municipal wastewater treatment plant that receives industrial pulp and paper wastewater proved to be an ideal inoculum for sulfate reducing EGSB reactors. Reactor A (Fig. 6) and Reactor B (Fig. 7) achieved 90.0% and 86.0% removal of sulfate, respectively, only 15 days after startup. This indicates the seed sludge contained SRB, and the SRB populations quickly acclimated to low a NaCl level (10 $g \cdot L^{-1}$). Starting a bioreactor with anaerobic methanogenic sludge and switching the bacterial community to an SRB dominated community can take months to years (Lens et al., 2002). This is especially true when common substrates for anaerobic species are used, such as hydrogen and acetate (Chen et al., 2008). In addition to a suitable inoculum, there are several physical and chemical factors that can be adjusted to promote the growth of SRBs, including pH (7-8), temperature (mesophilic conditions), COD/SO_4 ratio (<2.2), salinity and the electron donor (propionate).

B. Sulfate Reduction

Reactor A (the control at 15 $g \cdot L^{-1}$ NaCl) was able to operate with a sulfate removal efficiency of 98.0% observed at the beginning of the experiment on day 68 and in the later part of the experiment on day 170 (Fig. 8). The average sulfate removal efficiency outside of the shock period was 94.0 ± 2.59%. To observe the effects of increasing salinity, this sulfate reduction efficiency in Reactor A at 15 $g \cdot L^{-1}$ of NaCl was considered the baseline sulfate reduction performance for the experiment and was compared to the performance

of Reactor B under increasing salt concentrations. In Reactor B at 15 g·L^{-1} of NaCl, the average sulfate removal performance was 93.8 ± 1.3% (Fig. 9b). At the beginning of the experiment with salinity level 15 g·L^{-1} of NaCl, the sulfate removal efficiencies in both reactors were statistically similar with a p-value of 0.33.

In Reactor B, the system performance responded better to gradual acclimation of salinity by stepwise increasing the salinity from 15 to 35 g·L^{-1} of NaCl in 5.0 g·L^{-1} increments than by rapid acclimation. During the time period with a salt content of 20-30 g·L^{-1}, the total sulfide levels and the sulfate removal efficiencies were relatively constant, and were not greatly affected by stepwise increasing the NaCl levels. At a salinity level of 30 g/L of NaCl, Reactor B was still able to achieve a high average sulfate removal efficiency of 93.7 ± 1.2%. However, the system performance declined as the salinity increased to 35 g·L^{-1} of NaCl. Although 35 g·L^{-1} of NaCl was maintained for only 8 days, it appeared this salt content level began to inhibit system performance. When acclimation was tested by rapidly increasing the NaCl levels from 15 to 40 g·L^{-1}, salinity began to affect the system performance a week after of increasing the salinity. Several factors including salinity can shift a bacterial community, but this rapid increase in NaCl eventually overwhelmed the bacterial community to a point the sulfate removal efficiency dropped to 32.0% on day 104 (Fig. 9b). Based on the experimental results shown in **Figure 9b**, gradually increasing the NaCl every three weeks was an effective method to acclimate the granules to salinity. Excluding the data recorded at the salinity level of 35 g/L of NaCl, the sulfate reduction performance was not affected by salinity in the range of 15-30 g·L^{-1} of NaCl. In addition with a salinity range of 20-30 g·L^{-1} of NaCl, sulfate reduction efficiencies were > 90.0%, which were statistically similar to baseline sulfate

removal performance with a p-value of 0.46. Looking at Scheme I and Scheme II, one could conclude salinity inhibition begins to occur between 35-40 $g \cdot L^{-1}$. Of course, this is a case by case observation where other SRB strains might tolerate higher salinities, and other acclimation strategies to salinity might be successful. For example, Vallero et al. (2004) observed a different trend. A high sulfate removal rate of 3.7 g $SO_4^{2-} \cdot L^{-1} \cdot d^{-1}$ was observed 50 days after startup with an initial salt content of 50 $g \cdot L^{-1}$ of NaCl. Some possible explanations for the different observations may be linked to the biodiversity in the SRB species associated with the electron donor and number of substrates used.

C. VFA Comparison

Selecting a suitable electron donor is an important factor in designing a successful biological sulfate reduction system and favoring SRBs over other anaerobic species. In this paper, propionate was selected as the sole electron donor. Propionate was selected as the electron donor for a few reasons. First, little research has been conducted on sulfur conversion technologies using propionate as the sole electron donor. To the author's knowledge, this is the first-time propionate degradation in a sulfate reducing bioreactor treating a synthetic produced water has been investigated. Secondly, methanogens' low affinity towards propionate was an important consideration (Stams and Muyzer 2008). Using unadapted methanogen-dominated UASB granules, it is crucial to favor the growth of SRBs over other anaerobic microorganisms in as many ways as possible to decrease the acclimation period required for SRB. Lastly, the generation of alkalinity from the degradation of propionate can be very advantageous in sulfur conversion technologies, as it will reduce the toxicity of the dissociated and undissociated sulfide species produced during sulfate reduction.

Theoretically looking at the energetics for propionate degradation, SRB should outcompete other anaerobic organisms for common substrates including acetate, hydrogen, and propionate. SRBs can be classified into two groups: ones that partially oxidize organic material to acetate and CO_2, and ones that completely oxidize organic material to CO_2. The metabolic pathway partially oxidizing the organic material to acetate is more likely to occur than completely oxidizing the organic material to carbon dioxide. This is because many SRB species have the ability to partially oxidize organic material to acetate (Laanbroak et al., 1984). In addition, O'Flaherty et al. (1998), found that propionate-utilizing SRBs have a higher affinity to sulfate than acetate-utilizing SRBs. This observation may be seen in this paper, where nearly complete sulfate reduction results in an accumulation of acetate in the system (Fig. 9b and Fig 10b). With these observations, it was assumed that the biological reduction of sulfate to sulfide coupled with the oxidation of propionate to acetate was the governing microbial pathway utilized by SRBs in this experiment. The applicable reaction, via Chen et al. (2017), is:

$$4C_3H_5O_2^- + 3SO_4^{2-} + 3H^+ = 4C_2H_3O_2^- + 3HS^- + 4CO_2 + 4H_2O$$

From day 107 till the end of the experiment in both sulfate reducing EGSB reactors (Fig. 10a and Fig. 11a), the propionate removal efficiencies were similar with an approximate removal efficiency of 70.0%. Even though the propionate conversion efficiencies were statistically similar in both reactors, the effluent acetate concentrations were significantly different with a p-value of 1.4×10^{-4}. See **Table A12** in the appendix for information on the Student t-test. At higher salinity levels from 20-35 $g \cdot L^{-1}$ in Reactor B, the acetate effluent concentration averaged 350 mg $COD \cdot L^{-1}$, which was lower than Reactor A's average acetate effluent concentration of 492 mg $COD \cdot L^{-1}$. It is possible that

the bacterial communities were different in the two reactors, which could result in different metabolisms being utilized. DNA sequencing is being performed to understand the community structure, but this work was not finalized in time for publication herein. For anaerobic organisms using VFAs, Soto et al. (1993) found that propionate-degrading species are more sensitive to Na^+ concentrations than acetate-degrading species. The higher salinity in Reactor B may have selected for acetate-degrading SRBs in this reactor, which would explain the lower concentrations of acetate in the effluent at the highest NaCl concentrations.

The potential downside of using organic acids such as propionate in sulfate reducing reactors is the relatively large COD amounts in the effluent. The maximum effluent acetate concentration in Reactor A was 622 mg $COD \cdot L^{-1}$ on day 145 (Fig. 10b). For municipal applications, this would be an issue in meeting COD effluent permits, but for the application of reusing the treated water during oil and gas operations, the issue is not as concerning as there are no COD limits specified for injection waters. Yet, a disinfectant should be added to the solution to prevent biofilm growth along the well. Having a low COD/SO_4 favors SRB growth over other anaerobic organisms (Weijma et al., 2002; Chen et al., 2008). Throughout the experiment, the COD/SO_4 ratio was 1.5-2.3. During startup, this ratio is important in favoring SRB growth over other anaerobic organisms, and in reducing the acclimation period for the SRB (Fig. 6 and Fig. 7). It has been observed that methanogens and SRB actively compete for acetate at COD/SO_4 values between 1.7-2.2, while COD/SO_4 values < 1.7 favor SRBs (Choi and Rim 1991). However, De Smul et al. (1999) observed high sulfate removal efficiencies at COD/SO_4 values between 3.6-4.6 using ethanol as the sole electron donor. Differences in results are largely due to

differences in the organic substrates utilized. Laanbroak et al. (1984) ranked SRB affinity to electron donors as the following: H_2 > propionate > other organic substrates; thus, higher COD/SO_4 may have been shown to be successful in operating sulfate reducing reactors simply because the SRB present have a higher affinity for the selected substrate than other anaerobic microorganisms.

Another factor influencing competition between anaerobic organisms is salinity. Methanogens have been found to be strongly inhibited at NaCl levels exceeding 10 g·L^{-1} (Kugelman and McCarty, 1965), while others state the IC_{50} for NaCl inhibition may be closer to 13-20 g·L^{-1} of NaCl (Lefebvre et al., 2006). In this study, with a relatively constant COD/SO_4, the electron donor and the high salinity concentration seemed to have a large impact on favoring SRBs population over other anaerobic microorganisms. Still, the relatively low COD/SO_4 was important and is believed to have contributed significantly to reducing the acclimation period during startup. Once the biological sulfate reduction to sulfide proceeds, the total sulfide levels will also play a role in the competition between anaerobic organisms.

D. Sulfide Analysis

At 15 g·L^{-1} of NaCl in Reactor A (Fig. 8a), the average total dissolved sulfide in the system was 320 ± 10 mg·L^{-1} of S^{2-}. This was considered the baseline sulfide concentration level. On day 132 (Fig. 8a), the maximum recorded total dissolved sulfide concentration in Reactor A was 350 mg·L^{-1} of S^{2-}. Reactor B also had a relatively high maximum total dissolved sulfide concentration of 340 mg·L^{-1} observed at 30 g·L^{-1} of NaCl on day 159. In literature, the toxicity of sulfides to SRB has been debated over the years. However, in recent years and in the majority of literature investigating sulfate reducing reactors

(Moosa et al., 2006; Chen et al., 2008; Pokorna et al., 2014), many agree that sulfide does in fact have a negative effect on the activity of SRB, especially undissociated sulfide (hydrogen sulfide).

Sulfide has been reported to inhibit microbial activity by a few mechanisms. The uncharged hydrogen sulfide molecule has the capability of diffusing across the cell membrane and inhibiting cell activity. Sulfide inhibition may also occur from sulfide complexing with iron in the cytochromes, or with other metals, reducing the overall growth activity in the cell (Sánchez et al., 2014). Lopes et al. (2007) investigated the sulfide toxicity towards SRB in ethanol and acetate-fed sulfate reducing UASB reactors. At a constant pH of 7, they found that sulfide inhibition decreased the sulfate reduction efficiency to 35% after exposing the biomass for 70 days to a sulfide-rich medium containing 200 $mg \cdot L^{-1}$ of S^{2-}.To alleviate the sulfide toxicity, stripping via N_2 gas was used and proved to be an effective method in regaining system performance as a sulfate reduction efficiency of 96% was recorded a few days after stripping. Not only did N_2 stripping prove to be an effective method to remove hydrogen sulfide, but it also indicated that the sulfide toxicity was reversible.

It has been suggested that sulfide inhibition is mainly due to undissociated sulfide, and that extent of inhibition occurs faster and at lower concentrations with undissociated sulfide than with dissociated sulfide species (Moosa et al., 2006; Sánchez et al., 2014). The extent of the hydrogen sulfide toxicity is largely dependent on the system pH. At 30°C, the pKa for hydrogen sulfide is 7.0 in freshwater conditions; thus increasing the pH in the system can significantly reduce the impact of undissociated sulfide toxicity (Dries et al., 1998: Chen et al., 2008). With an average pH of 7.8 ± 0.2 and 7.9 ± 0.2 in the

effluent of Reactor A and Reactor B, respectively, the undissociated dissolved sulfide concentration is at a low enough concentration of <20 $mg \cdot L^{-1}$ that it does not pose a toxicity risk towards the SRB, since IC_{50} for hydrogen sulfide has been reported to be 30-250 $mg \cdot L^{-1}$ (Pokorna et al., 2014). Also, the type of biomass growth will influence the bacterial community's sensitivity to sulfide toxicity. Anaerobic granules operated in UASB and EGSB reactors allow the bacterial consortium to tolerate toxic compounds and other inhibiting substances to a greater extent than other systems, such as suspended growth systems (McHugh et al., 2003). Over the entirety of the experiment, sulfide inhibition was not observed in either reactor.

E. Salinity Shock from Rapidly Increasing Salinity to 40 $g \cdot L^{-1}$ of NaCl

This salinity stress test was performed to observe the effects on system performance and to observe the resiliency of the SRBs. The biomass in both Reactor A and Reactor B did not respond well to rapidly increasing the salt content from 15 $g \cdot L^{-1}$ to 40 $g \cdot L^{-1}$ of NaCl (Fig. 8 and Fig. 9). The degree of salinity inhibition on the system performance was much greater in Reactor B than Reactor A. This was due to the time period each salinity spike was maintained (Table 5). After increasing the salinity, the system performance did not decrease immediately, and the total sulfide level of 260 $mg \cdot L^{-1}$ of S^{2-} remained relatively constant 15 days after increasing the salinity content. After 21 days in Reactor B, salinity began to affect the SRB performance to where the total sulfide content was 90 $mg \cdot L^{-1}$ of S^{2-}, and the sulfate removal efficiency was 32%. In Reactor A (Fig. 8a), a similar observation was seen where after increasing the salinity in the system, the sulfide level declined to around 260-240 mg L^{-1} of S^{2-}. With the system performance in both reactors being statistically similar at the beginning of the experiment, the system performance in

Reactor A would have most likely decreased in a similar manner as observed in Reactor B if the time period was extended.

The purpose of rapidly increasing the salinity was to compare different schemes to acclimate granules to salinity. Gradually increasing the salinity proved to be an effective method to acclimate unadapted granules to salinity, which is in agreement with Omil et al. (1997). The other goal was to investigate the SRB resiliency to salinity changes. After the extended salinity shock and decrease in sulfate removal to 32%, the system was able to regain the baseline system performance in a matter of days after the NaCl concentration returned to 15 $g \cdot L^{-1}$. Ten days after the salinity shock (Fig. 9a), the total sulfide concentration increased from 90 $mg \cdot L^{-1}$ of S^{2-} to 320 $mg \cdot L^{-1}$ of S^{2-}, representing a 72% increase. These results show that salinity inhibition towards these SRB is reversible and that the SRBs present in the biomass are resilient to salinity changes.

F. Sulfide Removal Requirements before Injection

Typically, sulfate reduction is an undesired biotic reaction resulting in formation of H_2S. Hydrogen sulfide is very corrosive to concrete infrastructures and to metals commonly using in piping by the oil and gas industry. Thus, any hydrogen sulfide in the injection water could cause tremendous damage to surface and subsurface equipment and infrastructure. Not only is hydrogen sulfide an issue, but the total sulfide concentration could cause an array of issues, such as forming insoluble sulfide precipitates in the presence of metal ions (Fe, Al, Cu, and Cd) (La et al., 2003). For this biological sulfate reducing technology to be applicable in treating low salinity produced waters, sulfide must be removed before the water is injected.

In practice, the common sulfide management techniques include stripping, chemical precipitation, and biological oxidation (Lens et al., 2002). To ensure sulfate reduction is not affected by the sulfide removal step, a separate sequential phase may need to be added. The sulfide removal step was out of the scope of this research, but it is still of great importance in applying this technology for removing sulfate in produced water reuse applications. In the PHREEQC simulations, the sulfide was theoretically removed by chemical precipitation with the addition of a zinc salt to form insoluble ZnS precipitates. It was assumed the precipitates were settled out and the supernatant was extracted and used as the injection water.

G. PHREEQC

The importance of the PHREEQC simulations was to show the benefit and applicability of biologically reducing sulfate in produced waters with high calcium and sulfate. Oil production wells located in the Minnelusa formations are notorious for having production issues from sulfate scales due to the abundance of limy sandstones with secondary rocks including gypsum and anhydrite (Warren et al., 2010). The three produced waters simulated in the PHREEQC model all come from oil producing wells located in the Minnelusa formation. The three wells selected were distinct in their water composition parameters including Ca/SO_4 and salinity (Table 7).

Using the PHREEQC model assuming a temperature of 30°C and pressure of 1 atm, the saturation index (SI) values for gypsum and anhydrite in the synthetic produced water were -0.8 and -0.71, respectively (Fig 13a). These SI values verify that neither gypsum nor anhydrite will homogeneously precipitate at these conditions. In addition, this shows that the sulfate removals recorded throughout the experiment did in fact

result from the actual activity of the SRB and that the sulfate concentrations did not decrease because of calcium sulfate precipitation.

The main factors responsible in the formation of $CaSO_4 \cdot 2H_2O$ (gypsum) and $CaSO_4$ (anhydrite) include pressure, temperature changes, and dissolved ion concentrations of Ca^{2+} and SO_4^{2-} (Moghadasi et al., 2006). In Simulation I, the transition temperature for Well 3 at atmospheric conditions, was found to be at 48°C. With increasing pressure, the transition temperature increased (Fig. 12). In addition, the ionic strength will influence the transition temperature. As the ionic strength of the solution increases, the transition temperature between gypsum-anhydrite will decrease (Marshall and Slusher, 1964). Applying these concepts to the injection wells, anhydrite is most likely to be the stable phase at the bottom of the well, while gypsum is likely to form near the wellhead. This assumption is correct if only thermodynamic equilibria are considered; however, kinetics will also influence the stability of each phase. Anhydrite has the slowest formation kinetics of the two phases; thus it is typically found only at temperatures exceeding 120°C (Freyer and Voigt, 2003; Nasari et al., 2015).

In simulations I-IV, all saturation indices were negative indicating that no spontaneous precipitation would occur. However, PHREEQC calculates only the homogenous precipitation potential and does not account for heterogeneous precipitation. Heterogeneous is more likely to occur in nature and will form on surfaces such as wellheads, pipe walls, and material perforations. The activation energy needed in heterogeneous precipitation is much less than homogenous precipitation; thus, even a negative SI index may not rule out heterogeneous precipitation. Since the criteria to induce heterogeneous precipitation are complex in injection wells, past knowledge of

Well 3 having production issues from $CaSO_4$ scale was used to determine a theoretical threshold to induce heterogeneous precipitation. During injection periods, Well 3's produced water is blended with a nearby groundwater source (Table 8) at a 50% blending ratio. At surface conditions with a 50% blending ratio, the saturation index value for gypsum was determined to be -0.67. As a conservation measure, it was assumed that, for an injection well in the Minnelusa Oil formation, a saturation index value of ≤ -0.60 may induce heterogeneous precipitation for gypsum.

In **Table 7,** Well 3 is shown to produce moderately saline water (ionic strength = 1.96), Well 2 is near seawater salinity (ionic strength = 0.64), and Well 1 is a low salinity water (ionic strength = 0.54). Interestingly, the model estimated that Well 1 would have the highest gypsum and anhydrite scaling potential while Well 3 with a high salinity content had the lowest scaling potential among all three wells (Fig. 13). Based on the pressure and temperature ranges used in the PHREEQC model, the highest possibility of spontaneous formation of sulfate scale was at surface conditions. From an application perspective, at 30°C and at 1 atm, this was considered the worst-case scenario for the spontaneous formation of gypsum.

To assess the largest impact of minimizing the gypsum scaling potential by biologically reducing sulfate, simulation III was performed at surface conditions. In **Table 9**, it can be seen that Well 1 had the largest scaling potential for gypsum, and required greater removal of sulfate to remain below the theoretical threshold for heterogeneous precipitation. Although Well 1 is a low salinity produced water (NaCl ≈ 21 $g \cdot L^{-1}$), it still poses the highest risk for sulfate scaling which is attributable to its high sulfate concentration of 4,466 $mg \cdot L^{-1}$. For Well 3, with a relatively high TDS content, a

sulfate removal of 50% gave an SI value of -0.82, which is well below the theoretical threshold of -0.60. This observation underscores an important point, i.e., that high TDS does not necessarily mean higher scaling potential, which depends on the mineral formed and the activities of the matrix ions. Thyne and Brady (2016) also observed this trend in PHREEQC, that the TDS content in brine solutions did not greatly impact the precipitation potential for anhydrite. However, for the mineral halite, it was found that as the TDS increased, the precipitation potential also increased. Based on the salinity conditions tested in the sulfate reducing EGSB reactors and the gypsum scaling potential estimated using the PHREEQC model, the produced waters from Well 1 and Well 2 could greatly benefit from biological treatment via SRB.

Based on the sensitivity analysis to determine the conditions to induce the spontaneous formation of gypsum, Ca/SO_4 ratios were found to greatly influence the scaling potential for gypsum. It was observed (Fig. 15) that at higher Ca/SO4 values, the spontaneous precipitation of gypsum is more likely to take place. This relationship was consistent through a salinity range of 15-60 $g \cdot L^{-1}$. Yet Well 1, for which the Ca/SO_4 ratio was roughly 0.1, had the highest scaling potential among all three wells. The sulfate concentration used to generate **Figure 14** was 1622 $mg \cdot L^{-1}$. If the sulfate concentration was increased to the concentration observed in Well 1 of 4,466 $mg \cdot L^{-1}$, the lines would be shifted up and to left, resulting in SI values > 0 at much lower Ca/SO_4 values. Another observation from the sensitivity analysis was that as the salinity increased, the scaling potential for gypsum decreased. This inverse relationship between salt content (TDS) and scaling potential for gypsum was observed in each

simulation. Again, **Figure 15** supports the point that low salinity produced waters can still cause sulfate scaling issues.

Blending high TDS produced waters with fresh water is a common practice, but the overall benefits may not be substantial in the grand scheme of enhancing oil production. In addition, transporting and piping freshwater can be quite expensive, especially in water-stricken areas. In Simulation IV, involving blending a local groundwater source with produced water, the scaling potential of the system actually increased after blending. As shown in **Table 10**, the calcite scaling potential almost doubled from no blending to blending the produced water with groundwater at an 85% ratio. This makes sense because the groundwater source had a relatively high concentration of bicarbonate, almost 1000 $mg \cdot L^{-1}$. The scaling potential of gypsum did decrease, but the change was not significant. Comparing the gypsum scaling potentials at 0% and 85% blending ratios, the percent reduction in the scaling potential for gypsum was only 18%. One of the main mechanisms in the production of scale is the mixing of incompatible waters; thus, careful consideration of the water chemistry is essential in successfully managing scale formation and maintaining an efficient oil well. Blending freshwater with produced water can be an effective method for reducing the TDS and the scaling potential in a system if proper measures have been accounted for.

Conclusion

Sulfate-Reducing EGSB Reactors

Two sulfate-reducing EGSB reactors were successfully inoculated with municipal granular sludge that required only 15 days to achieve a 90% sulfate removal efficiency. Reactor A was the control specimen for the experiment, with a baseline added salt content of 15 $g \cdot L^{-1}$ of NaCl. The baseline sulfate removal and baseline total dissolved sulfide in Reactor A were 94.0 ± 2.6% and 321 ± 12 $mg \cdot L^{-1}$ of S^{2-}, respectively. Two schemes were investigated to acclimate the granules in Reactor B to salinity. Rapidly increasing the salt content from 15 to 40 $g \cdot L^{-1}$ of NaCl resulted in poor system performance, while stepwise increasing the salinity in 0.5 $g \cdot L^{-1}$ increments proved to be an effective acclimation method. At a salt content of 30 $g \cdot L^{-1}$, Reactor B was able to achieve a high average sulfate removal of 94.0%. A two-sample Student t-test between the baseline sulfate reduction in Reactor A and the sulfate reduction in Reactor B gave a p-value of 0.46. It can be concluded that in the range of 15-30 $g \cdot L^{-1}$ of NaCl, the sulfate reduction performance was not affected. Salt addition began to affect the system performance in terms of the sulfate reduction and the amount of sulfide produced when the salt dose increased to 35-40 $g \cdot L^{-1}$ of NaCl. However, the effects of the salt on the SRB were reversible, and the system was able to regain performance in 10 days with a total sulfide concentration of 320 $mg \cdot L^{-1}$ of S^{2-}.

Sulfide toxicity was not encountered throughout the entirety of the experiment, which could be attributed to the influent pH being at 7.5 and to the alkalinity generated from the degradation of propionate. Since the reactor pH was > 7.5 in both reactors, the undissociated sulfide concentration was low enough (< 25 $mg \cdot L^{1-}$) that it did not pose a

toxicity risk towards the SRB. The downside of using organic acids such as propionate in sulfate reducing reactors is the relatively large COD in the effluent from partial oxidation of propionate to acetate. For the application of reusing treated produced water during oil and gas operations, high COD in effluent is not concerning as in municipal applications, but bacterial growth should be minimized along the well depth using a disinfectant.

PHREEQC Model

PHREEQC modeling results indicate that produced water with low to moderate salinity (20-31 $g \cdot L^{-1}$ of NaCl), from wells tapping Wyoming's Minnelusa formation, may be susceptible to heterogeneous calcium sulfate precipitation. A high TDS water did not necessarily reflect a high calcium sulfate scaling potential. Overtime, the TDS in the reservoir may change and even increase; thus, having an SRB population resilient to salinity changes is ideal from an application perspective. From the sensitivity analysis, the aqueous sulfate concentration and the Ca/SO_4 ratio are better indicators of gypsum scaling potential. In addition, an increase in TDS resulted in a decrease in the gypsum scaling potential.

Blending freshwater with produced water can be an effective method for reducing the TDS content in the water, but as seen in the model results, blending of incompatible waters can actually increase the scaling potential of the system. In addition, economic and state pressures to use less freshwater and to reuse more flowback and produced water make blending an undesired treatment alternative. Based on the gypsum scaling potential estimated from the PHREEQC model, produced waters with low to moderate salinity (20-35 $g \cdot L^{-1}$ of NaCl) but high calcium and sulfate concentrations could greatly

benefit from a biological sulfate-reduction process. A process operated to remove only 75% of the sulfate removal would still significantly reduce the gypsum scaling potential of the injection water.

In conclusion, to the author's knowledge, this was the first-time sulfate reducing EGSB reactors were investigated for treatment of saline water containing salts other than NaCl, such as KCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6H_2O$, to simulate a synthetic produced water. The biological sulfate reduction process was able to achieve high levels of sulfate reduction (>90.0%) at low to moderate salinity levels (10-35 $g \cdot L^{-1}$ of NaCl). Based on calculations made using the PHREEQC model, oil production sites considering reusing produced waters commonly encountered in the Wyoming's Minnnelusa oil formation could greatly benefit from this technology. Future work in this area should focus on removing the sulfide content, and possibly considering the simultaneously removal of COD to prevent biofilm growth in wells and reservoirs. In addition, the cost savings associated from biological treatment to membrane filtration should be investigated further to quantify the cost differences between the two treatment alternatives.

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Appendix

Table A1. pH values for Reactor A

Reactor A Effluent	Reactor A Influent
8.05	
7.88	7.47
8.02	7.50
7.54	7.45
7.80	7.7
7.68	7.48
7.58	

Table A2. pH values for Reactor B

Reactor B Effluent	Reactor B influent
7.73	7.71
7.91	7.53
7.63	7.75
7.71	7.26
8.20	7.34
8.14	7.36
8.07	7.76
7.85	7.47
8.11	7.5
7.66	7.48
7.55	7.70

Table A3. Minimum detection limit data for acetate.

Acetate Standard, 5ppm	Acetate Peak Area	Adjusted Acetate Peak Area
5_1	475974	428,253
5_2	475324	445,860
5_3	407774	375,798
5_4	398305	379,787
5_5	447226	411,838
5_6	480717	443,625
5_7	417595	388,378
5_8	409525	380,504
5_9	380464	341,704
	Average peak area	399,527
	Standard Deviation	35,090

Table A4. Sulfate removal data for Reactor A.

Day	Sulfate Removal (%)	Influent Sulfate Concentration ($mg \cdot L^{-1}$)
67	98%	967
72	90%	1024
74	95%	1055
77	95%	1080
80	93%	1008
82	92%	1079
83	89%	1010
86	93%	1071
88	93%	1036
95	90%	1090
100	90%	1167
102	78%	1166
104	93%	1139
107	91%	1095
109	72%	1109
118	92%	1033
125	89%	1091
128	92%	1024
132	94%	1092
139	96%	951
142	95%	1001
145	97%	1001
153	95%	946
157	98%	996
159	94%	964
163	95%	957
165	94%	954
170	98%	1043
176	94%	441

Table A5. Sulfate removal data for Reactor B.

Day	Sulfate Removal (%)	Influent Sulfate Concentration ($mg \cdot L^{-1}$)
67	93%	967
72	95%	1024
74	95%	1055
77	93%	1080
80	92%	1008
82	84%	1041
83	84%	1038
86	86%	1050
88	84%	1031
95	76%	1110
100	51%	1167
102	37%	1166
104	32%	1139
107	90%	1095
109	71%	1109
118	94%	1088
125	94%	1093
128	94%	1010
132	95%	984
139	95%	983
142	96%	1010
145	96%	1010
153	96%	983
157	94%	1013
159	94%	979
163	94%	1013
165	93%	938
170	95%	923
172	92%	904
178	89%	997
181	85%	1028
183	84%	1041
185	83%	1035

Table A6. Two sample Student t-tests for comparing sulfate removal recorded from day 43 to day 80 in Reactors A and B with 15 g·L^{-1} NaCl.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0.947399	0.951765
Variance	0.000248	0.000636
Observations	9	9
Pooled Variance	0.000442	
Hypothesized Mean Difference	0	
df	16	
t Stat	-0.44033	
P(T<=t) one-tail	0.332795	
t Critical one-tail	1.745884	
P(T<=t) two-tail	0.665589	
t Critical two-tail	2.119905	

Table A7. Two sample Student t-tests for comparing sulfate removal recorded from day 115 to day 177 in Reactor A and B. with 20-30 g·L^{-1}of NaCl in Reactor B and 15 g·L^{-1} in Reactor A.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0.944349846	0.945136
Variance	0.000145162	0.000584
Observations	14	14
Pooled Variance	0.000364427	
Hypothesized Mean Difference	0	
df	26	
t Stat	-0.108961731	
P(T<=t) one-tail	0.457034723	
t Critical one-tail	1.70561792	
P(T<=t) two-tail	0.914069447	
t Critical two-tail	2.055529439	

Table A8. Two sample Student t-tests for comparing sulfate removal recorded from day 115 to day 185 in Reactor A and B. with 20-35 $g \cdot L^{-1}$ of NaCl in Reactor B and 15 $g \cdot L^{-1}$ in Reactor A.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	0.923672317	0.945136
Variance	0.001819975	0.000584
Observations	18	14
Pooled Variance	0.001284252	
Hypothesized Mean Difference	0	
df	30	
t Stat	-1.680757858	
P(T<=t) one-tail	0.05159728	
t Critical one-tail	1.697260887	
P(T<=t) two-tail	0.10319456	
t Critical two-tail	2.042272456	

Table A9. VFA data for Reactor A.

Day	Propionate removal (%)	Prop., mg/L	Prop., mg COD/L	Influent Sulfate, mg/L	COD/SO_4	Acetate, mg/L	Acetate, mg COD/L	Total COD in effluent, mg/L
72	70.67	1572	2059	1024	2.0	192	156	760
74	72.47	1274	1669	1055	1.6	199	162	622
77	69.54	1126	1475	1080	1.4	204	166	615
86	74.19	1843	2415	1071	2.3	98	80	703
88	60.02	1067	1398	1036	1.3	411	334	893
95	63.83	1258	1648	1090	1.5	180	146	742
100	69.35	1579	2069	1167	1.8	422	343	977
102	50.08	1484	1944	1166	1.7	566	460	1431
107	70.04	1230	1611	1095	1.5	172	140	623
118	63.77	1359	1780	1033	1.7	212	172	817
132	73.18	1768	2316	1092	2.1	524	426	1047
139	70.81	1632	2138	951	2.2	750	610	1234
142	59.93	1701	2228	1001	2.2	691	562	1455
145	72.79	1901	2490			765	622	1300
153	69.73	1506	1973	946	2.1	716	582	1180
157	76.21	1226	1606	996	1.6	629	512	894
159	70.98	1474	1931	964	2.0	529	430	990
165	69.26	1497	1962	954	2.1	582	473	1076
176	72.48	1254	1643			655	533	985

Table A10. VFA data for Reactor B.

Day	Propionate removal (%)	Prop., mg/L of	Prop., mg/L of COD	Influent Sulfate	COD/SO_4	acetate, mg/L	acetate, mg /L of COD	Total COD in effluent
72	73.89	1416	1855			160	130	614
74	71.27	1100	1441			220	179	593
77	67.89	1234	1616	1126	1.4	248	201	720
86	63.57	1436	1882	1050	1.8	508	413	1098
88	50.83	1219	1597	1031	1.5	455	370	1155
95	63.54	1296	1698	1110	1.5	425	346	965
100	60.47	1579	2069	1167	1.8	209	170	988
102	35.41	1484	1944	1166	1.7	116	95	1350
107	64.38	1402	1836	1095	1.7	343	279	933
118	71.04	1369	1793	1088	1.6	394	320	840
132	69.39	1820	2384	984	2.4	369	300	1030
139	67.20	1505	1972	983	2.0	409	332	979
142	69.11	1638	2146	1010	2.1	369	300	963
145	73.23	1583	2074	1010	2.1	320	261	816
153	68.70	1645	2155	983	2.2	598	487	1161
157	67.35	1241	1626	1013	1.6	355	289	820
159	72.17	1298	1701	979	1.7	398	324	797
163	62.55	1244	1629	1013	1.6	521	424	1034
165	72.40	1679	2200	938	2.3	399	324	932
178	61.70	1378	1806			434	353	1044

Table A11. Two sample student t-test for comparing propionate removal efficiencies recorded from day 107 to day 178 in Reactor A and B. with 20-35 g·L^{-1} of NaCl in Reactor B and 15 g·L^{-1} in Reactor A.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	70.14419883	67.90432623
Variance	26.81167515	15.02312139
Observations	13	13
Pooled Variance	20.91739827	
Hypothesized Mean Difference	0	
df	24	
t Stat	1.248608142	
P(T<=t) one-tail	0.111925745	
t Critical one-tail	1.71088208	
P(T<=t) two-tail	0.223851491	
t Critical two-tail	2.063898562	

Table A12. Two sample student t-test for comparing acetate effluent concentrations recorded from day 107 to day 178 in Reactor A and B. with 20-35 g·L^{-1} of NaCl in Reactor B and 15 g·L^{-1} in Reactor A.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	527.6923251	339.3014386
Variance	5369.970629	4595.322977
Observations	9	10
Pooled Variance	4959.863049	
Hypothesized Mean Difference	0	
df	17	
t Stat	5.82196381	
P(T<=t) one-tail	1.02E-05	
t Critical one-tail	1.739606726	
P(T<=t) two-tail	2.04082E-05	
t Critical two-tail	2.109815578	